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Reference Material

Botany Paper – I Section A

Microbiology Plant Pathology Cryptogams

(Updated 2017 Edition)

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Botany Paper – I Section A: Reference Material

Microbiology, Plant Pathology and Cryptogams

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PART – I

MICROBIOLOGY

Structure and reproduction / multiplication of viruses

An introduction to viruses

A virus is a small, ultramicroscopic, sub-cellular infectious agent which is obligate intracellular parasite. It means that a virus replicates only inside the living cells of other organisms. Viruses can infect all types of life forms, from animals and plants to bacteria and archaea.

Viruses exist in active form only inside a living cell. Outside a cell, a virus is largely inert. In this state it is known as a virion or a virus particle.

Virions have a very simple structure and they consist of two or three parts:

1. The genetic material made from either DNA or RNA
2. A protein coat that protects these genes
3. An envelope of lipids that surrounds the protein coat when they are outside a cell (only in some cases)

Discovery of viruses

Dmitri Ivanovsky, in 1892, described a non-bacterial pathogen infecting tobacco plants. Later the discovery of the Tobacco Mosaic Virus was made by Martinus Beijerinck in 1898.

In 1898, Friedrich Loeffler and Paul Frosch found evidence that the cause of foot-and-mouth disease in livestock was an infectious particle smaller than any bacteria.

Martinus Beijerinck in 1898 observed that the non-bacterial pathogen infecting tobacco plants multiplied only in cells that were dividing, but as his experiments did not show that it was made of particles, he called it a *contagium vivum fluidum* (soluble living germ) and re-introduced the word virus. Beijerinck maintained that viruses were liquid in nature, a theory later discredited by Wendell Stanley, who proved they were particulate.

The first images of viruses were obtained upon the invention of electron microscopy in 1931 by the German engineers Ernst Ruska and Max Knoll. In 1935, American biochemist and virologist Wendell Meredith Stanley examined the tobacco mosaic virus and found it was mostly made of protein. A short time later, this virus was separated into protein and RNA parts.

The tobacco mosaic virus was the first to be crystallised and its structure could therefore be elucidated in detail.

The first X-ray diffraction pictures of the crystallised virus were obtained by Bernal and Fankuchen in 1941. On the basis of her pictures, Rosalind Franklin discovered the full structure of the virus in 1955. In the same year, Heinz Fraenkel-Conrat and Robley Williams showed that purified tobacco mosaic virus RNA and its protein coat can assemble by themselves to form functional viruses.

Salient features of the viruses

- A virus is a small parasite that cannot reproduce by itself. Once it infects a susceptible cell, however, a virus can direct the cell machinery to produce more viruses.
- About 5,000 viruses have been described in detail so far, although there are millions of different types.
- Viruses are found in almost every ecosystem on Earth.
- The shapes of viruses range from simple helical and icosahedral forms to more complex structures. The average virus is about one one-hundredth the size of the average bacterium. Most viruses are too small to be seen directly with an optical microscope.
- Most viruses have either RNA or DNA as their genetic material.
- The nucleic acid may be single- or double-stranded.
- The entire infectious virus particle, called a virion, consists of the nucleic acid and an outer shell of protein.
- The simplest viruses contain only enough RNA or DNA to encode four proteins. The most complex can encode 100 – 200 proteins.

- The nucleic acid of a virion is enclosed within a protein coat, or capsid, composed of multiple copies of one protein or a few different proteins, each of which is encoded by a single viral gene.
- A capsid plus the enclosed nucleic acid is called a nucleocapsid.
- The host range — the group of cell types that a virus can infect — is generally restricted. It serves as a basis for classifying viruses. A virus that infects only bacteria is called a bacteriophage, or simply a phage. Viruses that infect animal or plant cells are referred to generally as animal viruses or plant viruses.
- A few viruses can grow in both plants and the insects that feed on them. An example is **Potato Yellow Dwarf Virus**, which can grow in leafhoppers (insects that feed on potato plant leaves) as well as in potato plants.
- Wide host ranges are characteristic of some strictly animal viruses, such as **Vesicular Stomatitis Virus**, which grows in insects and in many different types of mammalian cells.
- Viruses spread in many ways; viruses in plants are often transmitted from plant to plant by insects that feed on plant sap, such as **aphids**; viruses in animals can be carried by blood-sucking insects. These disease-bearing organisms are known as **vectors**. Influenza viruses are spread by coughing and sneezing. **Norovirus and rotavirus**, common causes of viral gastroenteritis, are transmitted by the faecal-oral route and are passed from person to person by contact, entering the body in food or water. HIV is one of several viruses transmitted through sexual contact and by exposure to infected blood.
- During active infection, the genome of most DNA-containing viruses that infect eukaryotic cells is transported (with some associated proteins) into the cell nucleus, where the cellular DNA is also found. Once inside the cell, the viral DNA interacts with the host's machinery for transcribing DNA into mRNA. The viral mRNA that is produced then is translated into viral proteins by host-cell ribosomes, tRNA, and translation factors.
- **Viral growth cycles are classified as Lytic or Lysogenic.**
- During lytic replication, host-cell ribosomes and enzymes are used to express viral proteins, which then replicate the viral genome and package it into viral coats. The multiple progeny virions produced within a single infected cell eventually are released, following cell lysis or gradual disintegration of the cell.
- In lysogeny, the viral genome is integrated into host-cell chromosomes, forming a prophage that is replicated along with the host genome causing no immediate harm. When suitably activated, a prophage enters the lytic cycle.

Virus taxonomy

Virus taxonomy involves naming and placing viruses into a taxonomic system. Currently about 5,000 types of virus have been described in detail and at least about 3000 more are believed to exist.

Due to the **pseudo-living nature of viruses** and very poor knowledge of viral evolution and phylogeny, virus classification is currently a subject of ongoing debate and proposals.

Viral taxonomy has progressed in two stages:

1. *The stage of classical approach during which the Linnaean Principles were followed:* In 1962, André Lwoff, Robert Horne, and Paul Tournier were the first to develop a scientific means of virus classification, based on the Linnaean hierarchical system. This system divided viruses in phylum, class, order, family, genus, and species, according to their shared properties and the type of nucleic acid forming their genomes.

The proposals also included in the classical phase of viral taxonomy are:

- a. **Holmes Classification (1948) : Based on viral host**
- b. **Casjens and Kings Classification (1975): Based on type of nucleic acid, presence of envelope, symmetry and site of assembly**

2. *The stage of Modern Classification:* The modern classification started in 1971 with the Baltimore System. It divides viruses into seven groups depending on a combination of their nucleic acid (DNA or RNA), strandedness (single-stranded or double-stranded), Sense, and method of replication. The Baltimore Group of Viruses are:

- I: **dsDNA viruses** (e.g. Adenoviruses, Herpesviruses, Poxviruses)
- II: **ssDNA viruses (+)sense DNA** (e.g. Parvoviruses)
- III: **dsRNA viruses** (e.g. Reoviruses)
- IV: **(+)ssRNA viruses (+)sense RNA** (e.g. Picornaviruses, Togaviruses)

V: (-)ssRNA viruses (-)sense RNA (e.g. Orthomyxoviruses, Rhabdoviruses)

VI: ssRNA-RT viruses (+)sense RNA with DNA intermediate in life-cycle (e.g. Retroviruses)

VII: dsDNA-RT viruses (e.g. Hepadnaviruses)

The International Committee on Taxonomy of Viruses (ICTV) began to devise and implement rules for the naming and classification of viruses early in the 1990s, an effort that continues to the present day.

Viral classification under ICTV system starts at the level of order and follows as thus, with the taxon suffixes given in italics:

Order (-virales)

Family (-viridae)

Subfamily (-virinae)

Genus (-virus)

Species

In the current (2008) ICTV taxonomy, five orders have been established, the *Caudovirales*, *Herpesvirales*, *Mononegavirales*, *Nidovirales*, and *Picornavirales*. The committee does not formally distinguish between subspecies, strains, and isolates. In total there are 5 orders, 82 families, 11 subfamilies, 307 genera, 2,083 species and about 3,000 types yet unclassified.

The taxonomy of viruses will remain a challenge for the biologists even in the decades to come. This would mainly be due to continuing discovery of new viruses and poor understanding of viral phylogeny.

Virus structure

A fully assembled infectious virus is called a **virion**. The simplest virions consist of two basic components: nucleic acid (single- or double-stranded RNA or DNA) and a protein coat, the capsid, which functions as a shell to protect the viral genome from nucleases and which during infection attaches the virion to specific receptors exposed on the prospective host cell. Capsid proteins are coded for by the virus genome. Because of its limited size, the genome codes for only a few structural proteins (besides non-structural regulatory proteins involved in virus replication). Capsids are formed as single or double protein shells and consist of only one or a few structural protein species.

Some virus families have an additional covering, called the envelope, which is usually derived in part from modified host cell membranes. Viral envelopes consist of a lipid bilayer that closely surrounds a shell of virus-encoded membrane-associated proteins. The exterior of the bilayer is studded with virus-coded, glycosylated (trans-) membrane proteins. Therefore, enveloped viruses often exhibit a fringe of glycoprotein spikes or knobs, also called **peplomers**. In viruses that acquire their envelope by budding through the plasma or another intracellular cell membrane, the lipid composition of the viral envelope closely reflects that of the particular host membrane. The outer capsid and the envelope proteins of viruses are glycosylated and important in determining the host range and antigenic composition of the virion.

Helical Symmetry

In the viruses with helical symmetry, identical protein subunits (protomers) self-assemble into a helical array surrounding the nucleic acid, which follows a similar spiral path. Such nucleocapsids form rigid, highly elongated rods or flexible filaments; in either case, details of the capsid structure are often discernible by electron microscopy.

Tobacco Mosaic virus is an example of Helical Symmetry virus.

Tobacco Mosaic virus has a rod-like appearance with approximately 18 nm in diameter and 300 – 310 nm length, with a central hollow cavity (about 4 nm wide) and helical symmetry with a pitch of 2.3 nm.

Its virion is made from: 2134 molecules of capsomeres (each capsomere consists of 158 amino acids and its molecular wt. is ~ 17.5 KD) One molecule positive sense linear ssRNA as the genomic RNA, which is 6401

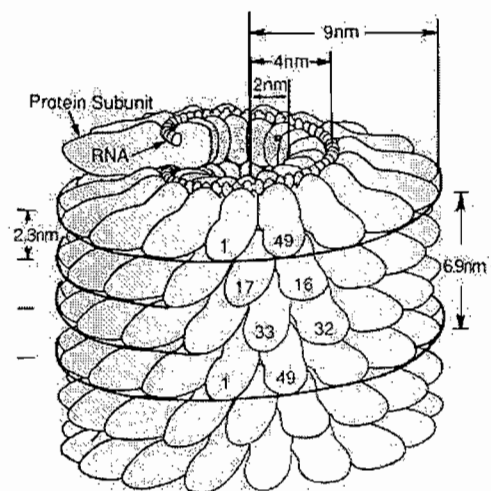


Figure: The helical structure of the rigid tobacco mosaic virus rod

bases long. This genomic RNA consists of only four genes – 1. gene for the coat protein 2. gene for the movement protein 3. gene for an RNA replicase 4. gene for a methyl / guanylyl transferase.

The capsomeres self assemble into the rod like helical structure (16.3 proteins per helix turn). The self assembly of the capsid starts with a particular site of the genomic RNA. This site is called the nucleation centre. In the final structure, the RNA is located helically at a radius of ~6 nm and is protected by the capsid proteins. There are about three RNA nucleotides per capsomere.

Icosahedral Symmetry

An icosahedron is a polyhedron having 20 equilateral triangular faces and 12 vertices. An icosaheron (polyhedral or spherical) with fivefold, threefold, and twofold axes of rotational symmetry is defined as having 532 symmetry (read as 5,3,2).

In most icosahedral viruses, the protomers, i.e. the structural polypeptide chains, are arranged in oligomeric clusters called capsomeres. The arrangement of capsomeres gives rise to an icosahedral shell.

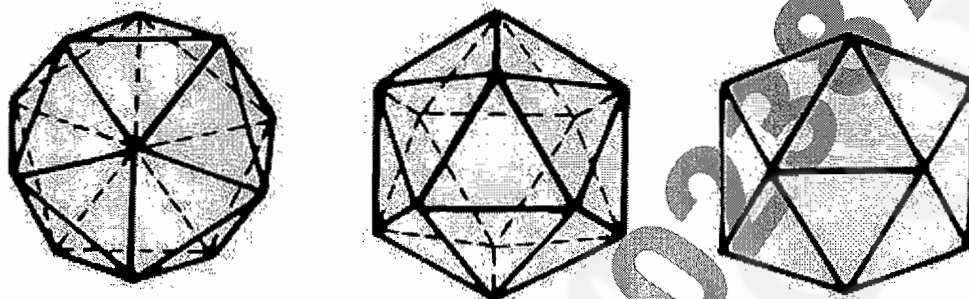


Figure: Icosahedral viral shell

Complex symmetry

It is a combination of elongated structure plus icosahedral structure. Further, certain additional components may also be seen. Bacteriophage T4 is a well studied example of complex symmetry virus.

Bacteriophage T4 is one of the largest of the bacterial viruses, about 300 nm long and therefore is an ideal structural model. The phage is represented schematically in the figure below.

T4 bacteriophage consists of

- an icosahedral head, where the DNA is stored
- a collar
- a contracting tail – which has an inner core surrounded by helical sheath
- a base plate
- six long and
- six tail spikes.

The long fibers recognize the host *E. coli* and make a loose attachment, and then the tail spikes fasten to get a tighter grip. The base plate is considered the nerve center for communicating between the spikes and the long fibers, and for regulating the interaction of tail fibers and the DNA injection machine.

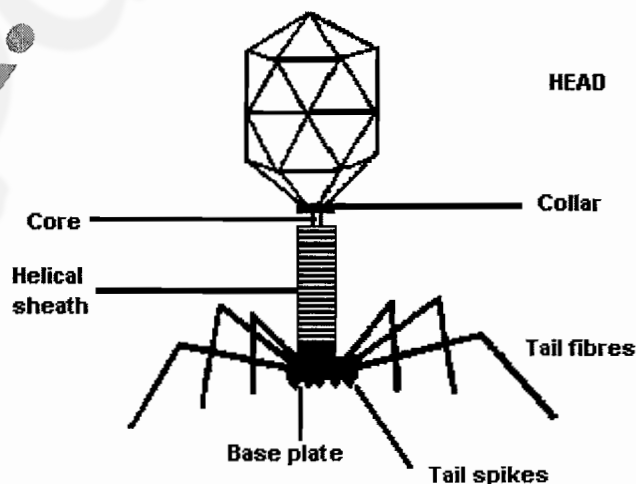


Figure: Bacteriophage T4 structure

Enveloped viruses

In addition to a capsid, some viruses have a modified form of the lipid bilayer membrane that is known as a viral envelope. This extra membrane is studded with proteins coded for by the viral genome and host genome, however the lipid membrane itself and any carbohydrates present in it are entirely host-coded.

The viral envelope can give a virion protection from enzymes and chemicals. The proteins studded upon the membrane (also called peplomers) includes glycoproteins functioning as receptor molecules, allowing healthy cells to recognise these virions as "friendly", resulting in the possible uptake of the virion into the cell. Some viruses are so dependent upon their viral envelope that they fail to function if it is removed. The

enveloped viruses possess a capsid which is neither purely helical, nor purely icosahedral. Moreover, it may possess extra structures such as protein matrix or a complex outer proteinaceous wall. The HIV is a good example of an enveloped virus.

Virus genetic material

The genome of a virus may consist of DNA or RNA, which may be single stranded (ss) or double stranded (ds), linear or circular. The entire genome may occupy either one nucleic acid molecule (monopartite genome) or several nucleic acid segments (multipartite genome). The different types of genome necessitate different replication strategies.

RNA Virus Genomes

RNA viruses, comprising 70% of all viruses, vary remarkably in genome structure. Because of the error rate of the enzymes involved in RNA replication, these viruses usually show much higher mutation rates than do the DNA viruses. Mutation rates of 10^{-4} lead to the continuous generation of virus variants which show great adaptability to new hosts.

The viral RNA may be single-stranded (ss) or double-stranded (ds), and the genome may occupy a single RNA segment or be distributed on two or more separate segments (segmented genomes). In addition, the RNA strand of a single-stranded genome may be either a sense strand (plus strand), which can function as messenger RNA (mRNA), or an antisense strand (minus strand), which is complementary to the sense strand and cannot function as mRNA protein translation.

The retrovirus genome comprises two identical, plus-sense ssRNA molecules, each monomer 7–11 kb in size, that are noncovalently linked over a short terminal region.

DNA Virus Genomes

Most DNA viruses contain a single genome of linear dsDNA. The papovaviruses, comprising the polyoma- and papillomaviruses, however, have circular DNA genomes, about 5.1 and 7.8 kb pairs in size. DsDNA serves as a template both for mRNA and for self-transcription.

Single-stranded linear DNA, 4–6 kb in size, is found with the members of the Parvovirus family that comprises the parvo-, the erythro- and the dependoviruses.

Circular single-stranded DNA of only 1.7 to 2.3 kb is found in members of the Circovirus family which comprise the smallest autonomously propagated viruses. The isometric capsid measures 17 nm and is composed of 2 protein species only.

Virus multiplication

The word reproduce is commonly used when discussing viruses, but in the strictest sense, viruses do not reproduce. Viruses use the machinery of the host cell to replicate themselves by creating an exact copy of the virus. Thus the viruses multiply using the host cell.

Viruses do not contain enzymes for energy production or protein synthesis.

For a virus to multiply, it must invade a host cell and direct the host's metabolic machinery to produce viral enzymes, viral proteins, and copies of its nucleic acid, using the host cell's ATP to power the reactions.

The surface of viruses includes many copies of one type of protein that binds, or adsorbs, specifically to multiple copies of a receptor protein on a host cell. This interaction determines the host range of a virus and begins the infection process. Then, in one of various ways, the viral DNA or RNA crosses the plasma membrane into the cytoplasm. The entering genetic material may be accompanied by inner viral proteins, although in the case of many bacteriophages, all capsid proteins remain outside an infected cell. The genome of most DNA-containing viruses that infect eukaryotic cells is transported (with some associated proteins) into the cell nucleus, where the cellular DNA is, of course, also found. Once inside the cell, the viral DNA interacts with the host's machinery for transcribing DNA into mRNA. The viral mRNA that is produced then is translated into viral proteins by host-cell ribosomes, tRNA, and translation factors.

After the synthesis of hundreds to thousands of new virions has been completed, most infected bacterial cells and some infected plant and animal cells rupture, or lyse, releasing all the virions at once. In many plant and animal viral infections, however, no discrete lytic event occurs; rather, the dead host cell releases the virions as it gradually disintegrates.

These events — adsorption, penetration, replication, and release — describe the lytic cycle of viral replication. The outcome is the production of a new round of viral particles and death of the cell. Figure below illustrates the lytic cycle for T4 bacteriophage.

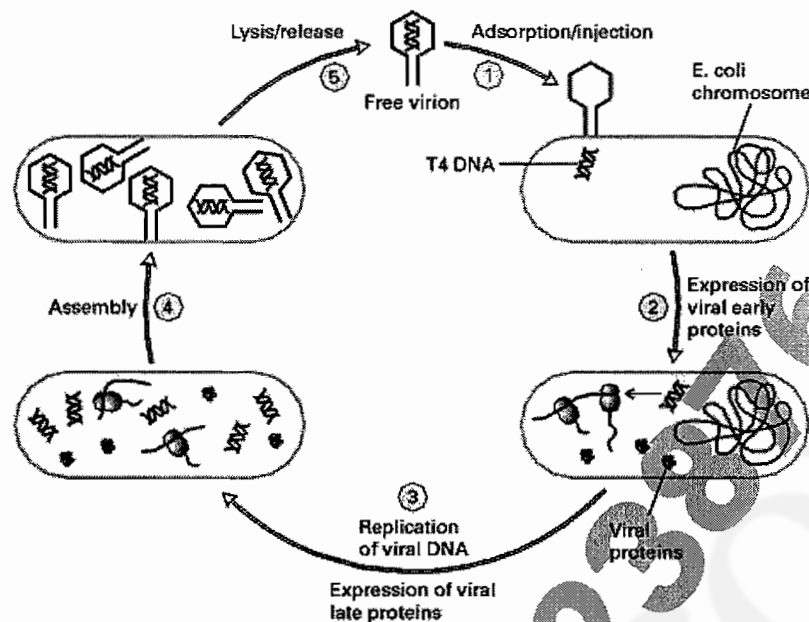


Figure: Lytic Pathway: During adsorption (step 1), viral coat proteins (at the tip of the tail in T4) interact with specific receptor proteins on the exterior of the host cell. The viral genome is then injected into the host. Next, host-cell enzymes transcribe viral "early" genes into mRNAs and subsequently translate these into viral "early" proteins (step 2), which replicate the viral DNA and induce expression of viral "late" proteins by host-cell enzymes (step 3). The viral late proteins include capsid and assembly proteins and enzymes that degrade the host-cell DNA, supplying nucleotides for synthesis of viral DNA. Progeny virions are assembled in the cell (step 4) and released (step 5) when the cell is lysed by viral proteins. Newly liberated viruses initiate another cycle of infection in other host cells.

In some cases, after a bacteriophage DNA molecule enters a bacterial cell, it becomes integrated into the host-cell chromosome, where it remains quiescent and is replicated as part of the cell's DNA from one generation to the next. This association is called **lysogeny**, and the integrated phage DNA is referred to as a prophage. Under certain conditions, the prophage DNA is activated, leading to its excision from the host-cell chromosome and entrance into the lytic cycle. Bacterial viruses of this type are called **temperate phages**. The genomes of a number of animal viruses also can integrate into the host-cell genome. The most important are the retroviruses.

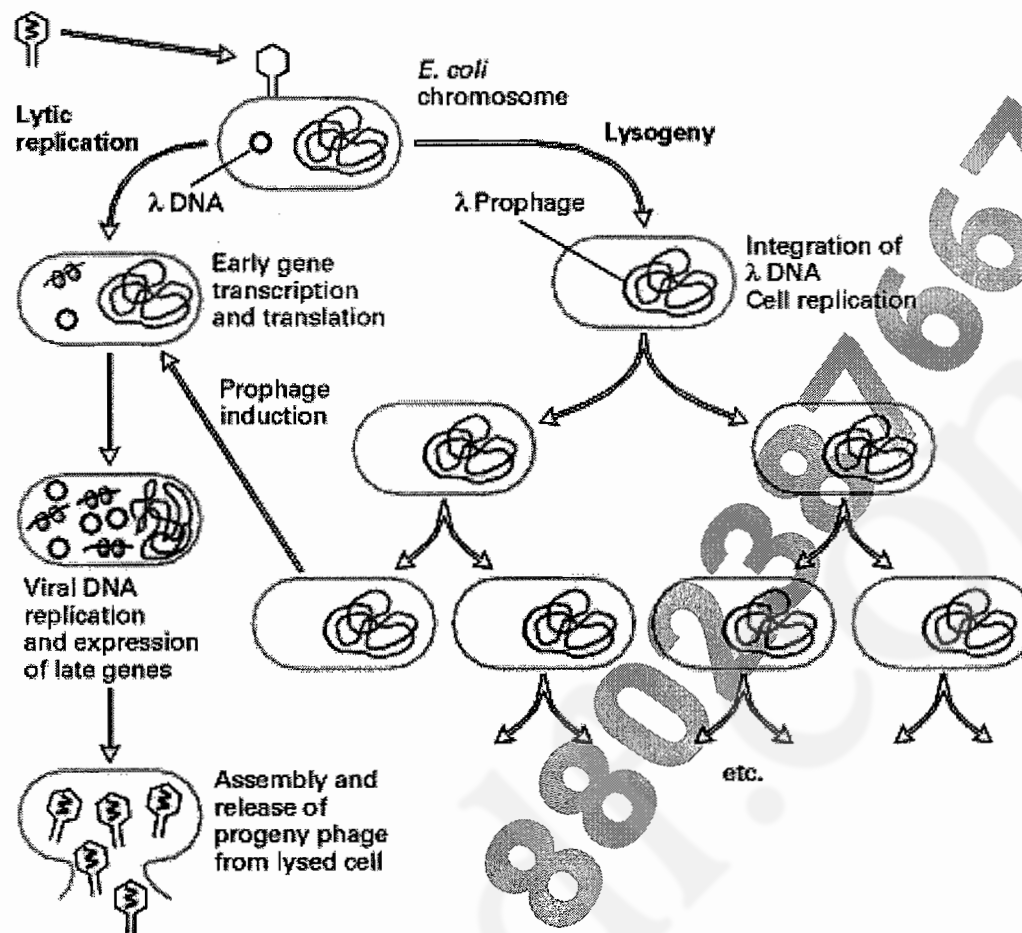


Figure: Lysogenic Pathway: The linear double-stranded DNA is converted to a circular form immediately after infection. (Left) If the nutritional state of the host cell is favorable, most infected cells undergo lytic replication, similar to lytic replication of cells by bacteriophage T4. (Right) If the nutritional state of the host cell cannot support production of large numbers of progeny phages, lysogeny is established. In this case, viral genes required for the lytic cycle are repressed, and host-cell enzymes synthesize viral proteins that integrate the viral DNA into a specific sequence in the host-cell chromosome where no host-cell genes are disrupted. The prophage DNA then is replicated along with the host-cell chromosome as the lysogenized cell (called a lysogen) grows and divides. Repression of the viral genes required for lytic replication is maintained in progeny cells. At infrequent intervals, the prophage in a lysogen is induced, or activated, leading to expression of viral proteins that precisely remove the prophage DNA from the host-cell chromosome and to derepression of the genes required for the lytic cycle. As a result, a normal cycle of lytic replication ensues.

Bacteriophages

Bacteriophages are the viruses which specifically parasitize bacterial cells. Mostly the term **Bacteriophages** does not include the **Cyanophages**, the viruses of **Cyanobacteria**, another group of prokaryotes.

Historical

Frederick Twort (1915) and **Felix d'Herelle (1917)** were the first to recognize viruses which infect bacteria, which d'Herelle called **bacteriophages** (eaters of bacteria).

In the 1930s and subsequent decades, pioneering virologists such as **Luria**, **Delbruck** and many others utilized these viruses as model systems to investigate many aspects of virology, including virus structure, genetics, replication, etc. These relatively simple agents have since been very important in the development of our understanding of all types of viruses, including those of man which are much more difficult to propagate and study. They are still a paradigm for many areas of biology, especially gene expression (For Example **Bacteriophage Lambda**).

Ecology

Bacteriophages, like bacteria, are very common in all natural environments and are directly related to the numbers of bacteria present. They are thus very common in soil and have shaped the evolution of bacteria.

Morphology & molecular structure

Bacteriophages range in size between 10 nm and 100 nm in width and between 10 nm and 1000 nm in length. The capsid symmetry can be:

1. Helical [enveloped or non enveloped]
2. Polygonal Isometric - [enveloped or non enveloped]
3. Polygonal Elongated - [enveloped or non enveloped]
4. Complex with elongated head – always non enveloped
5. Complex with isometric head – always non enveloped

Bacteriophage T4 is one of the largest of the bacterial viruses, about 300 nm long and therefore is an ideal structural model. The phage is represented schematically in the figure below.

T4 bacteriophage consists of

- an icosahedral head, where the DNA is stored
- a collar
- a contracting tail – which has an inner core surrounded by helical sheath
- a base plate
- six long and six tail spikes

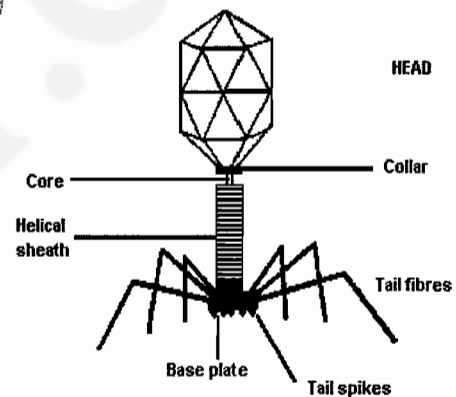


Figure: Bacteriophage T4

The long fibers recognize the host *E. coli* and make a loose attachment, and then the tail spikes fasten to get a tighter grip. The base plate is considered the nerve center for communicating between the spikes and the long fibers, and for regulating the interaction of tail fibers and the DNA injection machine.

Diversity

According to the Seventh Report of the ICTV, 1999, there are 16 distinct groups of bacteriophages, which are very diverse structurally and genetically. A description of phage diversity is presented in the figure below. Most bacteriophages [11 out of 16 Families] have dsDNA as genetic material.

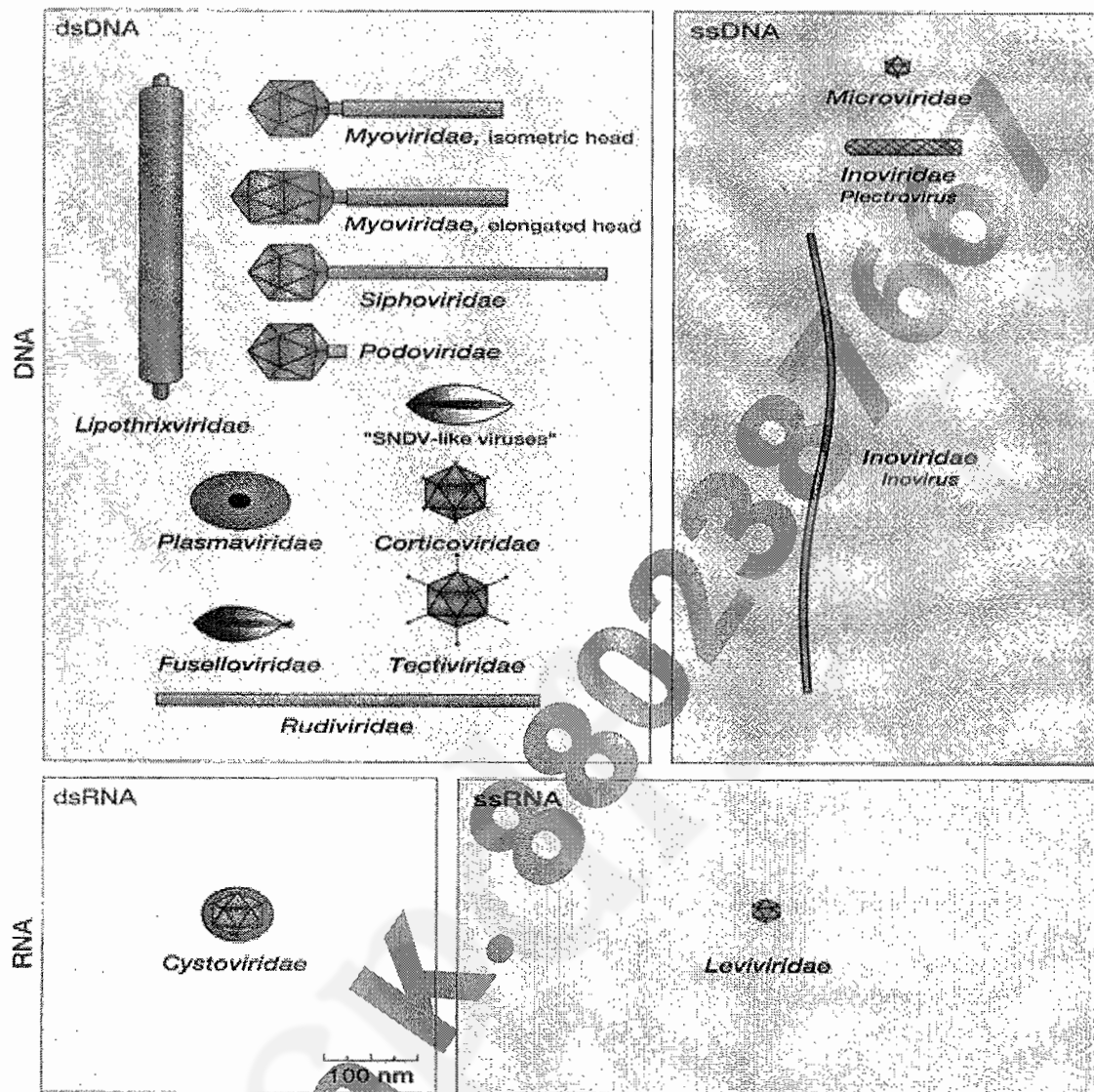


Figure: The diversity of Bacteriophages

Life cycle

There are two types of Bacteriophage life cycles:

1. **Lytic cycle:** Some phages on infecting a susceptible host, disturb its functions to the purpose of producing a large number of progeny phage particles. As the result of **lytic infection**, the host bacterium dies. In the typical lytic cycle, the phage DNA (or RNA) enters the host bacterium, its genes are transcribed in a set order, the phage genetic material is replicated, and the protein components of the phage particle are produced. Finally, the host bacterium is broken open (*lysed*) to release the assembled progeny particles by the process of **lysis**.

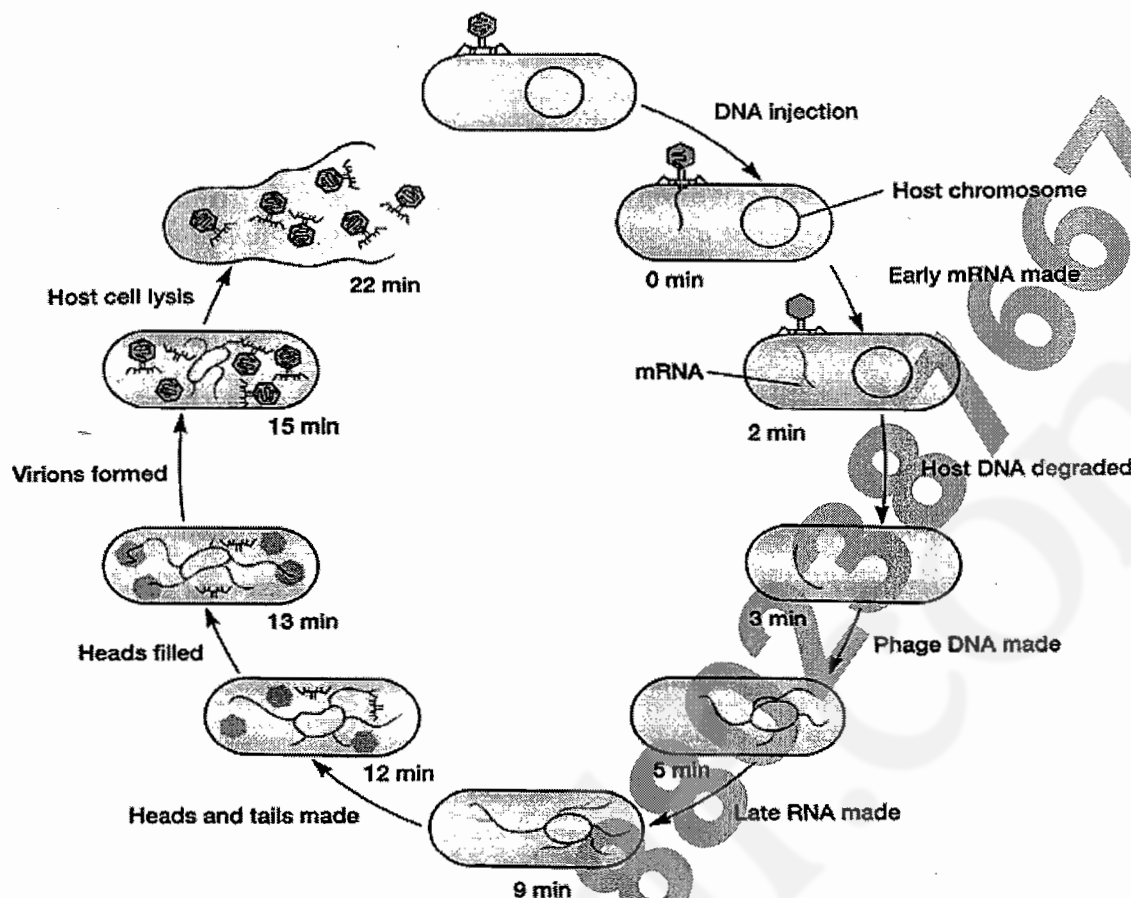
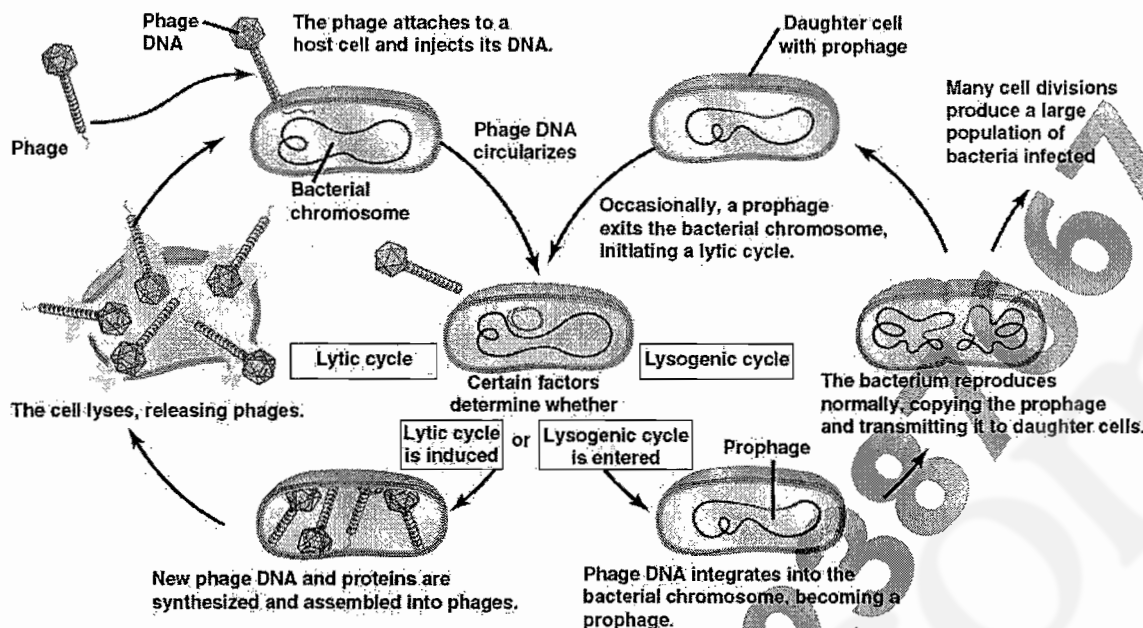


Figure: Lytic Cycle of T4 Phage

- Lysogenic Cycle:** Lysogeny is characterized by the fusion of the nucleic acid of a bacteriophage with that of a host bacterium. The newly integrated genetic material, called a prophage can be transmitted to daughter cells at each subsequent cell division, and a later event (such as UV radiation) can release it, causing proliferation of new phages via the lytic cycle.

The distinction between lysogenic and lytic cycles is that the spread of the viral DNA occurs through the usual prokaryotic reproduction, while the lytic phage is spread through the production of thousands of individual phages capable of surviving and infecting other cells. The key difference between the lytic cycle and the lysogenic cycle is that the **lysogenic cycle does not lyse the host cell**.

Phages that replicate only via the lytic cycle are known as **virulent phages** while phages that replicate using both lytic and lysogenic cycles are known as **temperate phages**. Temperate phages (such as **lambda phage**) can reproduce using the lytic or lysogenic cycle. Via the lysogenic cycle, the bacteriophage's genome is not expressed and is instead integrated into the bacteria's genome to form the prophage. Since the bacteriophage's genetic information is incorporated into the bacteria's genetic information as a prophage, the bacteriophage replicates passively as the bacterium divides to form daughter bacteria cells. In this scenario, the daughter bacteria cells contain prophage and are known as lysogens. Lysogens can remain in the lysogenic cycle for many generations but can switch to the lytic cycle at any time via a process known as **induction**. During induction, prophage DNA is excised from the bacterial genome and is transcribed and translated to make coat proteins for the virus and regulate lytic growth.



Significance

Phages of *Lactobacillus* are a serious problem for the dairy industry. In Medical sciences- phage typing (e.g. *Staphylococcus*); antibacterials are common practices. They have also been used as Recombinant DNA vectors - cloning, expression, and source of enzymes such as T4-DNA ligase.

Bacterial viruses have played a crucial role in the development of molecular cell biology and Four Types of Bacterial Viruses Are Widely Used in Biochemical and Genetic Research.

1. DNA Phages of the T Series: The initial discovery of the role of messenger RNA in protein synthesis was based on studies of *E. coli* cells infected with bacteriophage T2. By 20 minutes after infection, infected cells synthesize T2 proteins only.

2. Temperate Phages: Bacteriophage λ has one of the most studied genomes and is used extensively in DNA cloning. The carefully controlled action of viral genes makes λ DNA as a research material for studying regulation of gene expression.

3. Small DNA Phages: These small DNA phages are typified by the ϕ X174 and the filamentous M13 phages. These were the first organisms in which the entire DNA sequence of a genome was determined, permitting extensive understanding of the viral life cycle. They also have been particularly useful in identifying and analyzing the cellular proteins involved in DNA replication.

4. RNA Phages: Because their RNA genomes also serve as their mRNA, these phages are a ready source of a pure species of mRNA. In one of the earliest demonstrations that cell-free protein synthesis can be mediated by mRNA.

Plant Viruses

Introduction to viruses

A virus is a nucleoprotein that has the ability to cause disease. It multiplies only in living cells and it is too small to be seen individually with a light microscope. All viruses are parasitic in cells and cause a multitude of diseases in all forms of living organisms, from single-celled microorganisms to large plants and animals.

Although viruses behave like microorganisms in that they cause disease, have genetic functions, and are able to reproduce, they also behave as chemical molecules. At their simplest, viruses consist of nucleic acid and protein, with the protein forming a protective coat around the nucleic acid. Although viruses can take any of several forms, they are mostly either rod shaped or polyhedral, or variants of these two basic structures. There is always only RNA or only DNA in each virus and, in most plant viruses, only one kind of protein. Some viruses, however, may have two or more different proteins. Viruses do not divide and do not produce any kind of specialized reproductive structures such as spores. Instead, they multiply by inducing host cells to form more viruses. **Viruses cause disease not by consuming cells or killing them with toxins, but by utilizing cellular substances during multiplication, taking up space in cells, and disrupting cellular processes. These in turn upset the cellular metabolism and lead to the development of abnormal substances and conditions injurious to the functions and the life of the cell or the organism.**

The total number of viruses known to date exceeds 2000 and new viruses are described almost every month. About one fourth of all known viruses attack and cause diseases in plants. One virus may infect one or dozens of different species of plants and each species of plant is usually attacked by many different kinds of viruses. A plant may also be infected by more than one kind of virus at the same time.

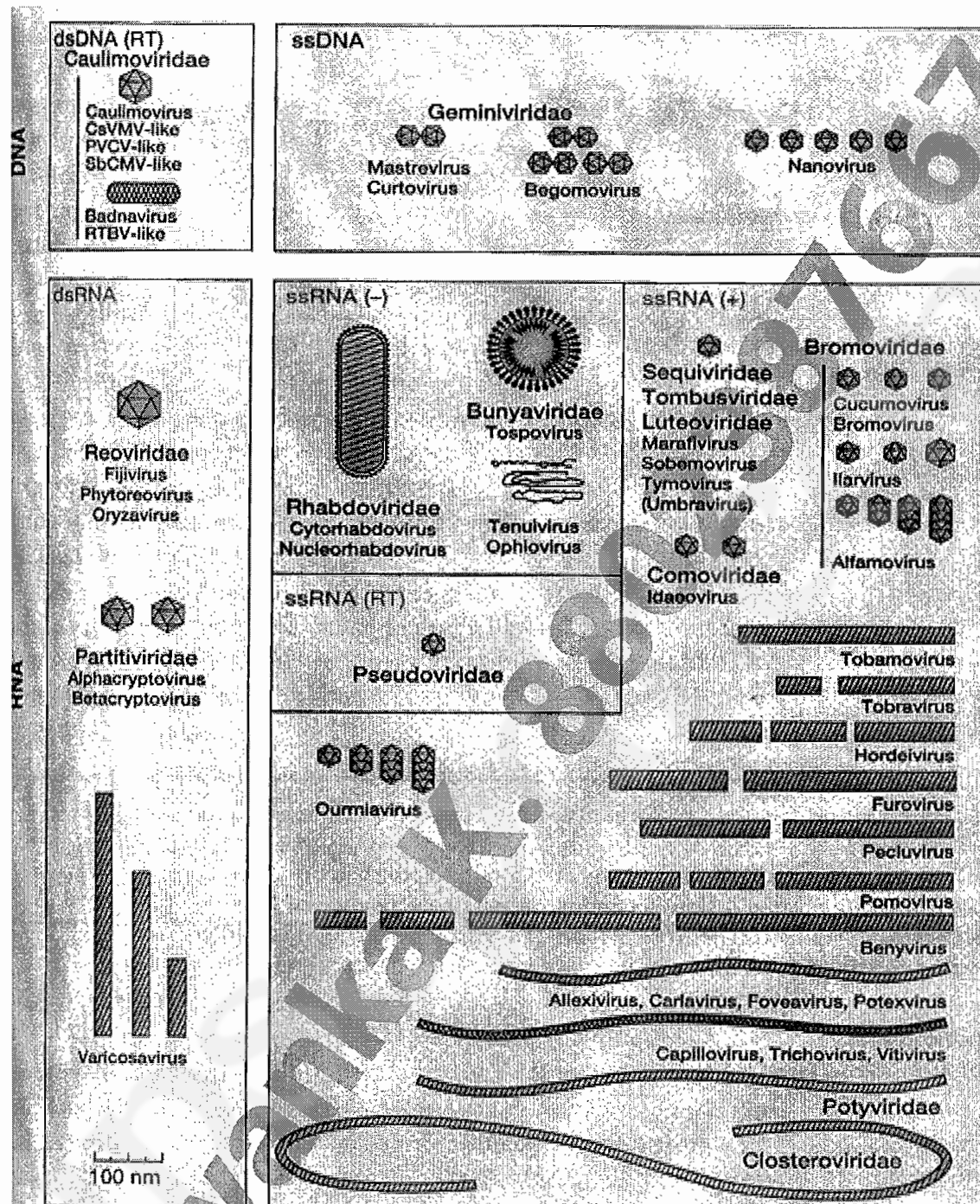
Characteristics of plant viruses

1. Plant viruses differ greatly from all other plant pathogens not only in size and shape, but also in the simplicity of their chemical constitution and physical structure, methods of infection, multiplication, translocation within the host, dissemination, and the symptoms they produce on the host.
2. Because of their small size and the fact that they are transparent, viruses generally cannot be viewed and detected by the methods used for other pathogens. Cell inclusions consisting of virus particles, however, are visible by light microscopy. **When a plant disease is caused by a virus, individual virus particles cannot be seen with the light microscope.**

Detection

1. **By observing inclusion bodies in the affected cells:** Frequently young leaf cells of virus-infected plants contain inclusion bodies of fairly distinctive shapes and sizes consisting of virus aggregates that can be seen with the light microscope and can be used to detect and identify the genus of the virus.
2. **By Electron Microscopy:** Examination of cell sections or of crude sap from virus-infected plants under the electron microscope may reveal virus like particles. **The most definitive proof of the presence of a virus in a plant is provided by purification, electron microscopy, and, most commonly, serology.**
3. **Symptomatic examination:** Most other symptoms caused by viruses resemble those caused by mutations, nutrient deficiencies or toxicities, insect or mite feeding damage, other pathogens, and other factors. However, a few plant symptoms can be attributed to viruses with a good degree of certainty, such as oak-leaf patterns on leaves and chlorotic or necrotic ring spots. The determination that certain plant symptoms are caused by viruses involves the elimination of every other possible cause of the disease. It also involves the transmission of the virus from diseased to healthy plants.

Diversity of plant viruses



Morphology, Composition and Structure

Plant viruses come in different shapes and sizes, which we can loosely arrange in 3 groups:

1. Nearly 50% of the plant viruses are **elongate** (rigid rods or flexuous threads) with dimensions in the range of 10 – 15 nm wide x 300 – 2000 nm long
2. About 45% many are **spherical** (isometric or polyhedral), with diameters in the range of 17 nm – 60 nm.
3. The remaining 5% are **cylindrical bacillus like** with dimensions in the range of 52-75 x 300-380 nm.

Each plant virus consists of at least a nucleic acid and a protein capsid. Some viruses consist of more than one size of nucleic acid and proteins, and some of them contain enzymes or membrane lipids. **The nucleic**

acid makes up 5 to 40 percent of the virus, protein making up the remaining 60 to 95 percent. The lower nucleic acid percentages are found in the elongated viruses, whereas the spherical viruses contain higher percentages of nucleic acid. The total mass of the nucleoprotein of different virus particles varies from 4.6 million to 73 million Da.

Genetic material: The nucleic acid of most plant viruses consists of RNA, but at least 80 viruses have been shown to contain DNA. In case of plant viruses the sequence and the frequency of the bases on the RNA strand vary from one RNA to another, but they are fixed within a given RNA and determine its properties.

Many plant viruses have **split genomes**. It means that they are consisting of two or more distinct nucleic acid strands encapsidated in different-sized particles made of the same protein subunits. This condition is called **Multipartite Virus Phenomenon** and it has been observed in a number of common viruses: e.g. 1. *Tobacco rattle virus* that consists of two rods, a long one (195 by 25 nm) and a shorter one (43 by 25 nm), 2. *Alfalfa mosaic virus*, consist of four components of different sizes.

Also, many **isometric viruses** or have two or three different components of the same size but containing nucleic acid strands of different lengths. In such multi component viruses all of the nucleic acid strand components must be present in the plant for the virus to multiply and perform in its usual manner.

Segmented virus genomes are those which are divided into two or more physically separate molecules of nucleic acid, all of which are then packaged into a single virus particle.

Capsid: The surface of viruses consists of a definite number of protein subunits, which are spirally arranged in the elongated viruses and packed on the sides of the polyhedral particles of the spherical viruses. In cross section, the elongated viruses appear as hollow tubes with the protein subunits forming the outer coat and the nucleic acid, also spirally arranged, embedded between the inner ends of two successive spirals of the protein subunits. In spherical viruses the visible shell consists of protein subunits, while the nucleic acid is inside the shell and is arranged in an as yet unknown manner.

Viral proteins, like all proteins, consist of amino acids. The sequence of amino acids within a protein, which is dictated by the sequence of nucleotides in the genetic material, determines the nature of the protein. The protein shells of plant viruses are composed of repeating subunits. The amino acid content and sequence for identical protein subunits of a given virus are constant but vary for different viruses and even for different strains of the same virus.

The content and sequences of amino acids are known for the proteins of many viruses. For example, the protein subunit of tobacco mosaic virus (TMV) consists of 158 amino acids in a constant sequence, and it has a mass of 17,600 Da or 17.6 kD. In TMV the protein subunits are arranged in a helix containing 16 1/3 subunits per turn (or 49 subunits per three turns). The central hole of the virus particle down the axis has a diameter of 4 nm, whereas the maximum diameter of the particle is 18 nm. Each TMV particle consists of approximately 130 helix turns of protein subunits. The nucleic acid is packed tightly between the helices of protein subunits.

Envelope: The rhabdoviruses, and a few spherical viruses, are provided with an outer lipoprotein envelope or membrane. Inside the membrane is the nucleocapsid, consisting of nucleic acid and protein subunits.

Multiplication of plant viruses

- Plant viruses which have RNA genome encode their own RNA replicase and make copies of their own genome within the host cell.
- DNA genome containing plant viruses use host cell's DNA polymerase enzyme to replicate their own genome.
- Capsid proteins are also encoded by the viral genome.
- Viral genome moves from one cell to another cell of the infected plant through **plasmodesmata**.

Classification of plant viruses

The Plant Virus Subcommittee of the International Committee on Nomenclature recommended a classification of viruses into 16 subgroups. These groups are:

1. Tobravirus group
2. Tobamovirus group
3. Potexvirus group
4. Carlavirus group
5. Potyvirus group
6. Cucumovirus group
7. Tymovirus group

8. Comovirus group
9. Nepovirus group
10. Bromovirus group
11. Tombusvirus group
12. Caulimovirus group
13. Alfalfa mosaic virus
14. Pea enation mosaic virus
15. Tobacco necrosis virus
16. Tomato spotted wilt virus

Satellite Viruses and Satellite RNAs

In addition to typical viruses, which consist of one or more rather large strands of nucleic acid contained in a capsid composed of one or more kinds of protein molecules, and which can multiply and cause infection by themselves, two other types of virus like pathogens are associated with plant diseases.

1. **The satellite viruses** are viruses but they must always be associated with certain typical viruses (helper viruses) because they depend on the latter for multiplication and plant infection. Satellite viruses often reduce the ability of the helper viruses to multiply and cause disease; that is, satellite viruses act like parasites of the associated helper virus.
2. **The satellite RNAs** are small, linear or circular RNAs found inside virions of certain multicomponent viruses. Satellite RNAs are not related, or are only partially related, to the RNA of the virus; satellite RNAs may increase or decrease the severity of viral infections.

Pathogenicity of plant viruses

One virus may infect one or dozens of different species of plants and each species of plant is usually attacked by many different kinds of viruses. A plant may also be infected by more than one kind of virus at the same time.

Plant viruses differ greatly from all other plant pathogens not only in size and shape, but also in the simplicity of their chemical constitution and physical structure, methods of infection, multiplication, translocation within the host, dissemination, and the symptoms they produce on the host.

Viruses cause many important plant diseases and are responsible for huge losses in crop production and quality in all parts of the world. Infected plants may show a range of symptoms depending on the disease but often there is leaf yellowing (either of the whole leaf or in a pattern of stripes or blotches), leaf distortion (e.g. curling) and/or other growth distortions (e.g. stunting of the whole plant, abnormalities in flower or fruit formation).

Transmission of Plant Viruses

The plant viruses rely on damage in the cell wall to enter into a plant cell. This is achieved either by the vector or simply by mechanical damage to cells. The main modes of plant virus transmission are as follows.

1. **Seeds:** Seeds formed by virus infected plants may contain viruses. These viruses are present in the next generation right since the beginning, leading to early outbreaks of disease in new crops. About 100 viruses are known to be transmitted this way.
2. **Pollens:** Pollens formed by infected flowers contain viruses. The viruses transmitted by this method result into low levels of fruit set. In some cases it may spread from the fertilized flower into the remaining body of the mother plant. Example: *Prunus necrotic ringspot virus*.
3. **Vegetative propagation/grafting:** If the vegetative propagation material is not virus free, the resulting plants will also be virus infected.
4. **Bacteria:** In case of *Agrobacterium tumefaciens*, the Ti plasmid of this organism has been used experimentally to transmit virus genomes between plants.
5. **Fungi Vectors:** Fungi can insert their hypha into the plant cell and transmit the virus also. About 15 plant viruses are transmitted with help of fungal genera like *Ophiostoma*, *Polymyxa* and *Spongospora*.
6. **Nematode Vectors:** - About 20 plant viruses are transmitted by nematodes, especially by the nematode genera: *Longidorus*, *Paralongidorus*, and *Xiphinema*. The important viruses transmitted by nematodes are: Grape fan leaf virus and Tobacco ringspot virus.
7. **Insect vectors:** It is a particularly efficient means of virus transmission. Insects, which bite or suck plant tissues transmit viruses to new hosts. This is known as *non-propagative transmission*. Group III

geminivirus are transmitted by insect vectors (leafhoppers or whiteflies) by this method. These viruses cause a great deal of crop damage in plants such as tomatoes, beans, squash, cassava and cotton.

However, in other cases (e.g. many plant *rhabdoviruses*) the virus may also infect and multiply in the tissues of the insect (*propagative transmission*) as well as those of host plants. In these cases, the vector serves as a means not only of distributing the virus, but also of amplifying the infection.

8. **Mite Transmission:** Mites belonging to the family Eriophyidae have been shown to transmit at least 6 known viruses including the Wheat streak mosaic virus.
9. **Mechanical Transmission:** Mechanical transmission of viruses is the most widely used method for experimental infection of plants and is usually achieved by rubbing virus-containing preparations into the leaves. This is also an important natural method of transmission. Virus particles may contaminate soil for long periods and may be transmitted to the leaves of new host plants as wind-blown dust or as rain-splashed mud.
10. **Dodder Transmission:** The parasitic plant *Cuscuta* is a major source of infection for host angiosperms. This is known to transmit at least 16 different viruses.

Control of Plant Viruses

Plant viruses cannot be directly controlled by chemical application. The major means of control (depending on the disease) include:

1. **Chemical or biological control of the vector** (the organism transmitting the disease, often an insect): This can be very effective where the vectors need to feed for some time on a crop before the virus is transmitted but are of much less value where transmission occurs very rapidly.
2. **Growing resistant crop varieties:** In some crops and for some viruses there are highly effective sources of resistance that plant breeders have been using for many years. Transgenic resistance has shown considerable promise for many plant-virus combinations. For example, the use of this approach in Hawaii to control *Papaya ringspot virus* has been credited with saving the local papaya industry.
3. **Use of virus-free planting material:** In vegetatively propagated crops (e.g. potatoes, many fruit crops) and where viruses are transmitted through seed major efforts are made through breeding, certification schemes etc., to ensure that the planting material is virus-free.
4. **Exclusion:** The prevention of disease establishment in areas where it does not yet occur. This is a major objective of plant quarantine procedures throughout the world as well as more local schemes.

Viroids

Introduction to viroids

Viroids are small, circular, single-stranded RNA molecules that cause several infectious plant diseases. They are called sub-viral agents because their structure is simpler than even a virus. They are also obligate intracellular parasite as they totally rely upon the host cell for multiplication.

Structure

The viroids are circular molecules of RNA. There is no protein associated in the structure of a viroid. This makes the viroids different from the viruses.

Under electron microscope, the viroids appear about 40 nm long and about 2.2 nm thick.

Their size ranges from between 246 – 375 nucleotides. Most of this RNA is unpaired, but some regions are double-stranded. The molecular weight of a viroid is between 107,000 and 127,000 Dalton.

H. J. Gross *et al.* sequenced the nucleotides of the Potato Spindle Tuber Viroid (PSTVd) in 1978. It consists of 359 ribonucleotides. It has numerous intra-molecular base-pairings which give stability to the structure.

The PSTVd structure resembles a dumb-bell. The functionally distinct parts of the viroid are called domains. The structure of potato spindle tuber viroid (PSTV) is indicated schematically below.

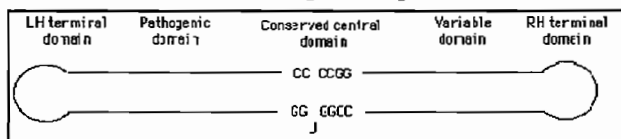


Figure: The structure of the PSTVd

Diversity

About 40 plant diseases are caused by 40 different viroids. There are two groups of viroids.

1. **Avsunviroid (ASBVd) group or Group A:** The viroids of this group lack the conserved central region of RNA, which is depicted in Figure 1. This group has only 4 viroids, including Avocado Sunblotch Viroid, Peach Latent Mosaic Viroid etc.
2. **Pospiviroid (PSTVd) group or Group B:** The viroids of this group contain the conserved central region of RNA, which is depicted in Figure 1. This group has 36 well characterized members. Members of this group include Potato Spindle Tuber Viroid, Coconut Cadang Cadang Viroid, Tomato Plant Macho Viroid, Citrus Bent Leaf Viroid, Pear Blister Canker Viroid etc.

Pathogenicity

Out of the 40 viroid diseases known in plants the most important diseases are:

1. Cadang cadang disease of coconut
2. Potato spindle tuber disease
3. Citrus exocortis
4. Avocado sunblotch
5. Apple scar skin
6. Chrysanthemum stunt

Pathogenicity is determined by the terminal and pathogenicity domains of the viroid.

The molecular basis of pathogenicity is unknown but it is suspected that viroid multiplication inhibits host's own metabolic processes such as transcription of genes. Molecular studies support that viroids are mostly located in the nucleus of the host cell, associated with chromatin. This might enable viroids to interfere with host gene expression.

Viroids are not known to encode any protein from their genome.

The transmission of viroids is also not well understood but it is based on insect and fungal vectors.

Multiplication

Viroids multiply even at relatively high temperature (about 35°C). Most likely, they have adapted to their host plants that have so far strictly been found to inhabit tropical, subtropical, and continental climates.

Viroid multiplication is not clearly understood yet. It does not carry or encode its own RNA replicase. Thus, it is dependent upon the RNA polymerase of the host cells.

Available evidences suggest the following mechanism of direct viroid multiplication using the RNA polymerase of host cell.

1. The circular viroid RNA (called + RNA) is replicated continuously to create multimeric linear complementary RNA (called — RNA).
2. The linear — RNA is replicated then. It produces multimeric +RNA.
3. The multimeric +RNA is processed then. In the processing cleaving of individual units take place and then circularization occurs.

The last step creates many copies of viroids.

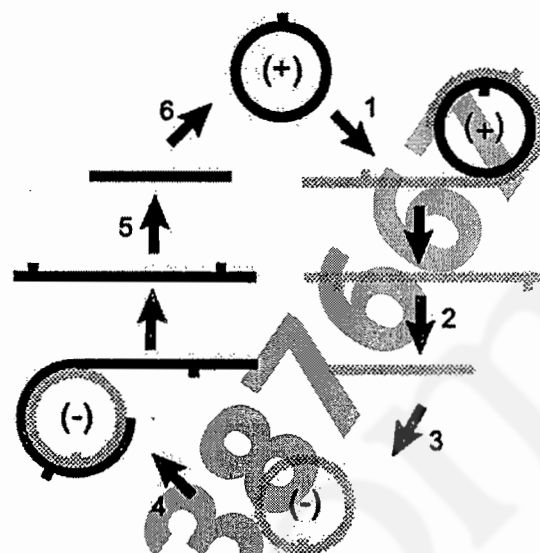


Figure: Viroid replication: Six separate steps: i.e., synthesis of multimeric (–)RNAs and (+)RNAs (steps 1 and 4), cleavage of these multimeric RNAs (steps 2 and 5), and circularization of the resulting linear (–)RNA and (+)RNA monomers (steps 3 and 6).

Control

The control of diseases caused by viroids is based on:

1. Use of viroid free propagating stock
2. Removal and destruction of viroid infected plant
3. Washing of hands and sterilizing of tools after handling viroid infected plants.

Virusoids

Virusoids or Satellite RNAs are also several hundred nucleotides long circular and single stranded RNA molecules, causing pathogenesis but not having any structural protein associated with them. They depend on a helper virus for replication. This helper virus also encapsidates them. e.g:

- Barley yellow dwarf virus satellite RNA: Helper – Luteovirus.
- Tobacco ringspot virus satellite RNA: Helper – Nepovirus
- Subterranean clover mottle virus satellite RNA: Helper – Sobemovirus

Two types of terminologies have been used for various satellite agents by plant pathologists. However, in strict sense it is only the satellite RNAs which can be actually regarded as virusoids.

1. **The satellite viruses** are viruses but they must always be associated with certain typical viruses (helper viruses) because they depend on the latter for multiplication and plant infection. Satellite viruses often reduce the ability of the helper viruses to multiply and cause disease; that is, satellite viruses act like parasites of the associated helper virus.
2. **The satellite RNAs** are small, linear or circular RNAs found inside virions of certain multicomponent viruses. Satellite RNAs are not related, or are only partially related, to the RNA of the virus; satellite RNAs may increase or decrease the severity of viral infections.

Virusoids replicate in the cytoplasm using an RNA-dependent RNA polymerase. This enzymic activity is common in plants but not found in animal cells.

Five virusoid RNA genomes are fully sequenced. They are 220–338 nucleotides long, circular, single stranded and possess a ribozyme activity.

These agents may modify the symptoms of infection by their helper virus. They do not interfere with the replication of their helper virus and are therefore differentiated from defective interfering particles that are associated with many viral infections.

The virusoids can be spread by any method by which the helper virus spreads for example: via insects, fungi, vegetative propagation, within seeds or by direct inoculation by man.

There are similar infectious agents which also infect animals, e.g. *Newt satellite 2 transcript*.

It is not known if viroids and virusoids are the progenitors of modern viruses or have degenerated from other more complicated viruses.

Prions & Prion Hypothesis

Prion -short for proteinaceous infectious particle (-on by analogy to virion) — is an infectious agent composed only of protein.) The prions are an abnormal form of a normally harmless proteins found in the brain of mammals. This abnormal form is responsible for a variety of fatal spongiform neurodegenerative disorders of both cattle and humans. These disorders are also called *transmissible spongiform encephalopathies*.

Prions have been held responsible for a number of degenerative brain diseases, including scrapie (a fatal disease of sheep and goats), mad cow disease, Creutzfeldt-Jacob disease, fatal familial insomnia, kuru, an unusual form of hereditary dementia known as Gertsman-Straussler-Scheinker disease, and possibly some cases of Alzheimer's disease.

History & discovery

Scrapie in sheep was first described during the 18th century. It has been transmitted to other animals such as mink and cats, and more recently to cows (mad cow disease or bovine spongiform encephalo-pathy, BSE) through contaminated feedstuff.

The first prion disease in humans that caught the attention of the physicians was Kuru, although initially the physicians could not identify it as a disease caused by a proteinaceous infectious agent. Kuru is a disease which affects the brain. It is endemic among the Fore tribe of Papua New Guinea and is universally fatal. For many years, physicians thought that various spongiform neurodegenerative diseases resulted from infection with *slow-acting viruses*, because of the lengthy incubation times required for the illnesses to develop, sometimes taking more than 30 years to display symptoms.

The pathogenic agent of these diseases have certain viral attributes, such as extremely small size and strain variation, but other properties are different from viruses. In particular, the agent is resistant to ultraviolet radiation, which normally inactivates viruses by destroying their nucleic acid.

In the early 1980s the American neurologist **Stanley B. Prusiner** and colleagues identified the "**proteinaceous infectious particle**," a name that was shortened to "**prion**". Prusiner was awarded the **Nobel Prize in Physiology or Medicine for 1997**, for his discovery of "prions - a new biological principle of infection".

Causation of prion disease

An unusual characteristic of prion diseases is that they can cause all the major forms of disease.

1. Through horizontal transmission (infection) from e.g. a sheep to a cow (BSE).
2. In inherited forms, mutations in the prion gene are transmitted from parent to child.
3. They can even arise spontaneously.

Once present in the brain prions multiply by inducing benign proteins to refold into the abnormal shape. This mechanism is not fully understood, but another protein normally found in the body may also be involved.

The normal protein structure is thought to consist of a number of flexible coils called alpha helices. In the prion protein, some of these helices are stretched into flat structures called beta [β] strands. The normal protein conformation can be degraded rather easily by cellular enzymes called proteases, but the prion proteins with predominantly β - conformation is more resistant to this enzymatic activity. Thus, on one hand prion proteins can multiply rapidly, they are not broken down by proteases and instead accumulate within nerve cells, destroying them.

Progressive nerve cell destruction eventually causes brain tissue to become filled with holes in a sponge like, or **spongiform**, pattern.

Diseases caused by prions that affect humans include:

1. **Creutzfeldt-Jakob disease**,
2. **Gerstmann-Sträussler-Scheinker disease**,
3. **Fatal familial insomnia**,
4. **Kuru**.

Prion diseases affecting animals include

1. **Scrapie**,
2. **Bovine spongiform encephalopathy** (commonly called mad cow disease),

3. Chronic wasting disease of mule deer and elk.

All known prion diseases are fatal. Since the immune system does not recognize prions as foreign, no natural protection develops. This happens because the Prion Proteins are modified forms of host body's own protein.

→ Prions affect different regions of the brain. A sponge-like appearance results when nerve cells die.

Symptoms depend on which region of the brain is affected.

1. **Cerebral cortex:** When the cerebral cortex is affected, the symptoms include loss of memory and mental acuity, and sometimes also visual impairment (CJD).
2. **Thalamus:** Damage to the thalamus may result in insomnia (FFI).
3. **Cerebellum:** Damage to the cerebellum results in problems to coordinate body movements and difficulties to walk (kuru, GSS).
4. **Brain stem:** In the mad cow disease (BSE), the brain stem is affected.

Mutations can result in different shapes of the prion protein that accumulates in different regions of the brain: In familial insomnia (FFI), mutated prions accumulate in the thalamus, with the result that the patients are unable to sleep. In Creutzfeldt-Jakob disease, the prion protein accumulates primarily in the cerebral cortex.

Mechanism of prion action

Route of infection

When cows are fed with offals prepared from infected sheep, prions are taken up from the gut and transported along nerve fibers to the brain stem. Here prions accumulate and convert normal prion proteins to the disease-causing form, PrP^{Sc}. Years later, BSE results when a sufficient number of nerve cells have become damaged, affecting the behaviour of the cows. The BSE epidemic reached its maximum in 1992 with 37,000 affected animals. When feedstuff containing sheep offals was banned, the incidence of BSE decreased rapidly. There is now a fear that humans might have been infected by eating products from BSE-affected cows giving rise to a new variant type of CJD.

The kuru epidemic started a long time ago and has subsided since the 1950s. The mad cow disease epidemic started in 1985 affecting approximately 170,000 cows. Kuru disappeared among the Fore-people when they stopped their cannibalistic rituals. In children the disease disappeared more rapidly, while adults continued to be affected. An incubation time of up to 30 years has been reported.

Molecular Mechanism

The prion protein exists in two forms. The normal, innocuous protein (PrP^C) can change its shape to a harmful, disease-causing form (PrP^{Sc}). The conversion from PrP^C to PrP^{Sc} then proceeds via a chain-reaction. When enough PrP^{Sc} proteins have been made they form long filamentous aggregates that gradually damage neuronal tissue. The harmful PrP^{Sc} form is very resistant to high temperatures, UV-irradiation and strong degradative enzymes.

The mechanism behind conformational change from PrP^C to PrP^{Sc} is not clearly understood till date. In 2005, Pei-Yeh Chen, Chun-Cheng Lin *et al* provided evidence that an aberrant N-linked glycosylation is responsible for the structural transition from PrP^C to PrP^{Sc}. This aberrant N-linked glycosylation occurs in the RER lumen affecting the 128 – 140 domain of the PrP^C.

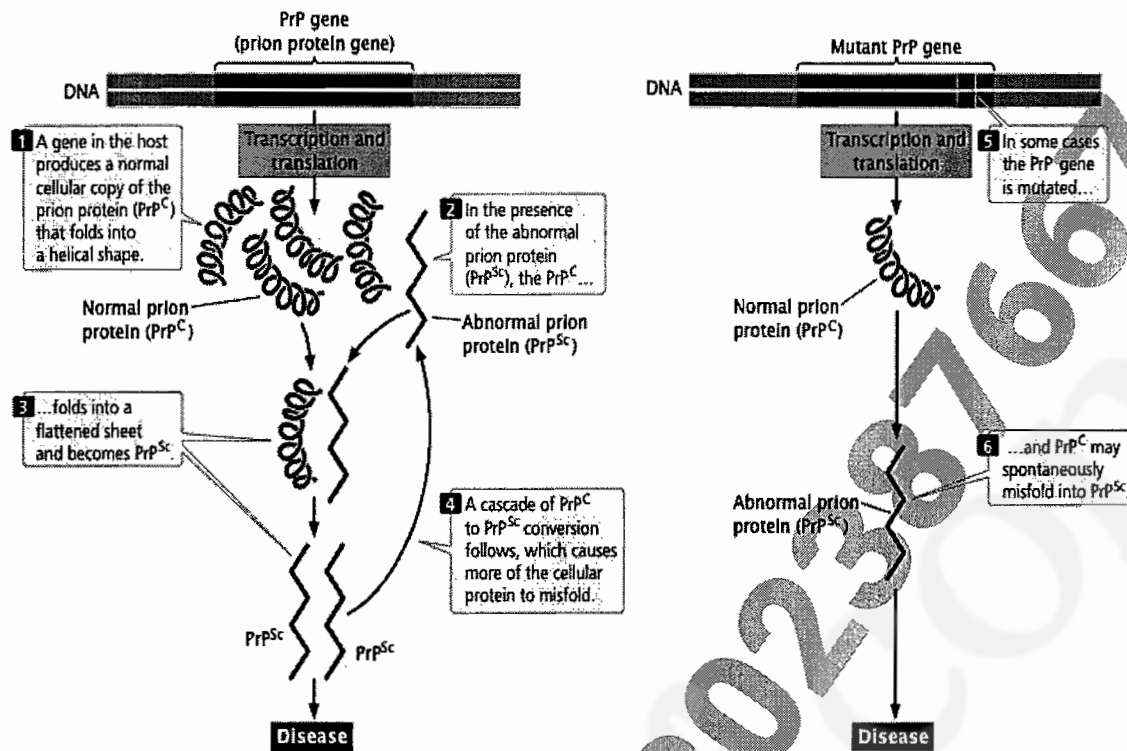


Figure: Prion Molecular Mechanism

Medical Efforts against Prion Diseases

All known prion diseases are untreatable and fatal.

However, a vaccine has been developed in mice that may provide insight into providing a vaccine in humans to resist prion infections.

Additionally, in 2006 scientists announced that they had genetically engineered cattle lacking a necessary gene (the *PRNP* gene) for prion production – thus theoretically making them immune to BSE, building on research indicating that mice lacking normally-occurring prion protein are resistant to infection by scrapie prion protein.

Bacteria: Structure & Multiplication

Introduction

The bacteria are the members of the domain **Bacteria**, according to the modern Three Domain Classification system. They are prokaryotic microorganisms, as their DNA is not enclosed within a nuclear system (Gr. *pro*, before; *karyote*, nucleus). They are very small [$<1 \mu\text{m}$ – $50 \mu\text{m}$ diameter] and single-celled or sometimes colonial.

The internal cell structure of the bacteria is relatively simple and most of the cellular complexity is associated with the cell surface structures. Bacteria lack membrane bound organelles as well as internal membrane structures such as the Endoplasmic Reticulum and Golgi Apparatus—typically associated with eukaryote cells. However, it is known now that some photosynthetic bacteria do have internal membranes (called Cytoplasmic Membrane System) which make the photosynthetic lamella. Similarly the magnetotactic bacteria (like *Aquaspirillum magnetotacticum*) have membrane enclosed magnetite (Fe_3O_4) particles called the Magnetosomes.

Bacterial cells have characteristic shapes (cocci, rods, spirals, etc.) and often occur in characteristic aggregates (pairs, chains, tetrads, clusters, etc.). These traits are usually typical for a genus and are diagnostically useful.

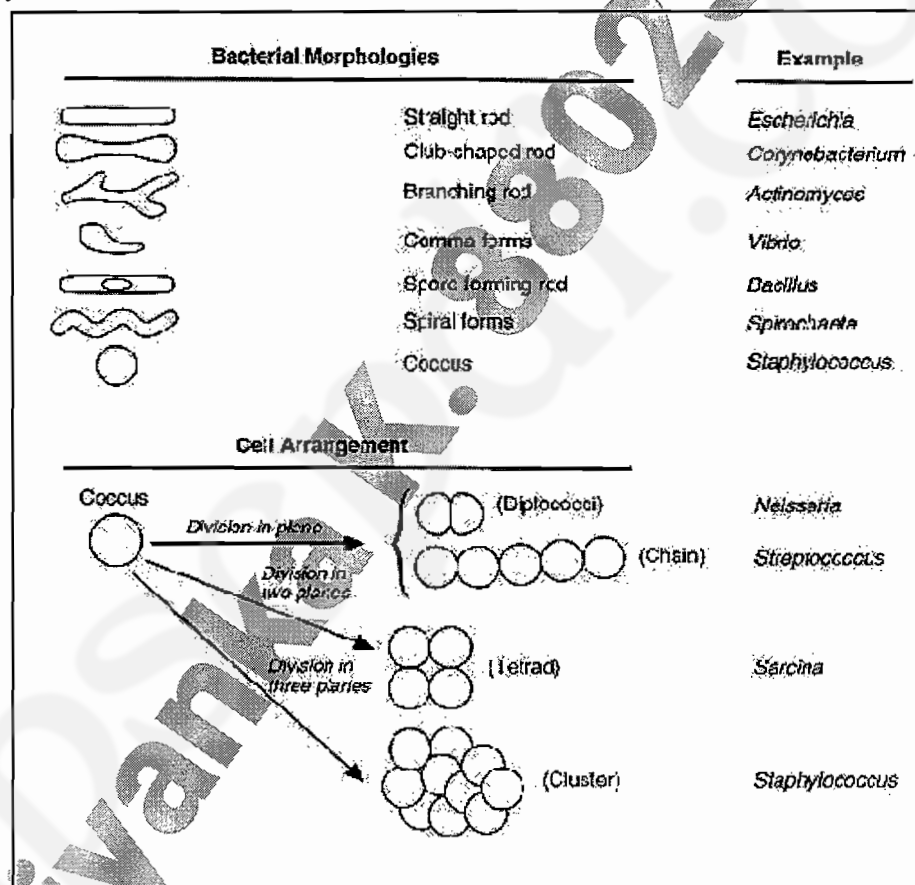


Figure 1: Figure: Various types of bacterial morphologies. Apart from the above morphological plans, there are some rare forms of morphologies. Examples of rare morphologies which are not shown in this figure include: Budding morphology (*Rhodospirillum*); Appendaged morphology (*Hyphomicrobium*) and Long filamentous morphology (*Chloroflexus*).

Cell Organization

Bacterial cells, which are essentially prokaryotic, have a nucleoid (nuclear body) rather than an enveloped nucleus and lack membrane-bound cytoplasmic organelles like the eukaryotic cells. The plasma membrane in prokaryotes performs many of the functions carried out by membranous organelles in eukaryotes.

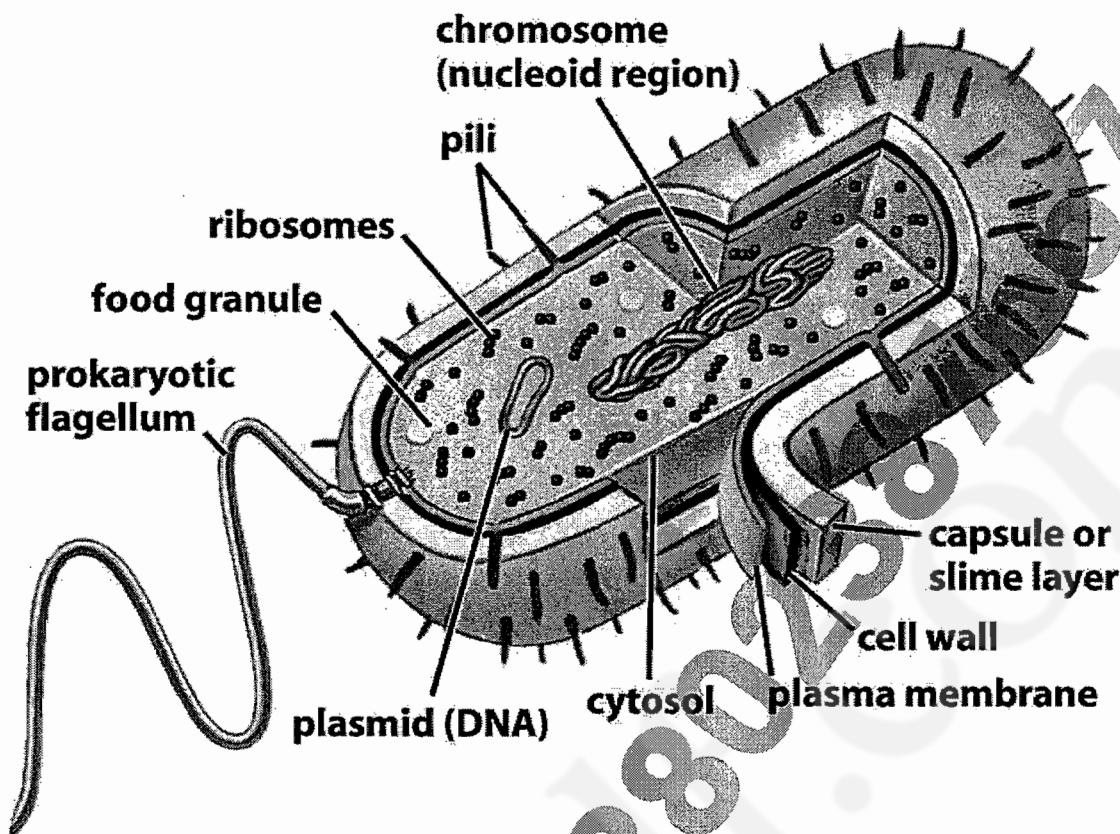


Figure: Basic organization of a bacterial cell.

Starting from the external surface, the following structures are found in bacterial cells as we move towards the centre of the cell. However, with individual species the details may vary somewhat.

Surface Appendages

Two types of surface appendage can be recognized on certain bacterial species: the flagella, which are organs of locomotion, and pili (*Latin for hairs*), which are also known as fimbriae (*Latin for fringes*). Flagella occur on both Gram-positive and Gram-negative bacteria, and their presence can be useful in identification. For example, they are found on many species of bacilli but rarely on cocci. In contrast, pili occur almost exclusively on Gram-negative bacteria and are found on only a few Gram-positive organisms (e.g., *Corynebacterium renale*).

Flagella

Structurally, bacterial flagella are long (3 to 12 μm), filamentous surface appendages about 12 to 30 nm in diameter. The protein subunits of a flagellum are assembled to form a cylindrical structure with a hollow core. A flagellum consists of three parts:

1. the long filament, which lies external to the cell surface; (Chemically, flagellar filaments are constructed of a class of proteins called **flagellins**. These proteins are spirally arranged around a hollow core. They do not show the 9+2 or 9+0 organization.)
2. the hook structure at the end of the filament; and

3. the basal body, to which the hook is anchored and which imparts motion to the flagellum. The basal body traverses the outer wall and membrane structures. It consists of a rod and two or four discs (two discs in Gram positive bacteria and four discs in Gram negative bacteria). In the basal body apart from the discs, there are also two major classes are movement related proteins:
 - a. **Mot Protein:** It is the motor protein and it links the energy input to the disc movement.
 - b. **Fli Protein:** It acts as the flagellar switch.

The hook and basal-body structures consist of numerous proteins.

The thrust that propels the bacterial cell is provided by counterclockwise rotation of the basal body, which causes the helically twisted filament to whirl. The movement of the basal body is driven by a proton motive force rather than by ATP directly. The ability of bacteria to swim by means of the flagella provides them with the mechanical means to perform chemotaxis (movement in response to attractant and repellent substances in the environment). Response to chemical stimuli involves a sophisticated sensory system of receptors that are located in the cell surface and/or periplasm and that transmit information to chemotaxis proteins that control the flagellar motor.

The number and distribution of flagella on the bacterial surface are characteristic for a given species and hence are useful in identifying and classifying bacteria. Figure below illustrates typical arrangements of flagella on or around the bacterial surface.

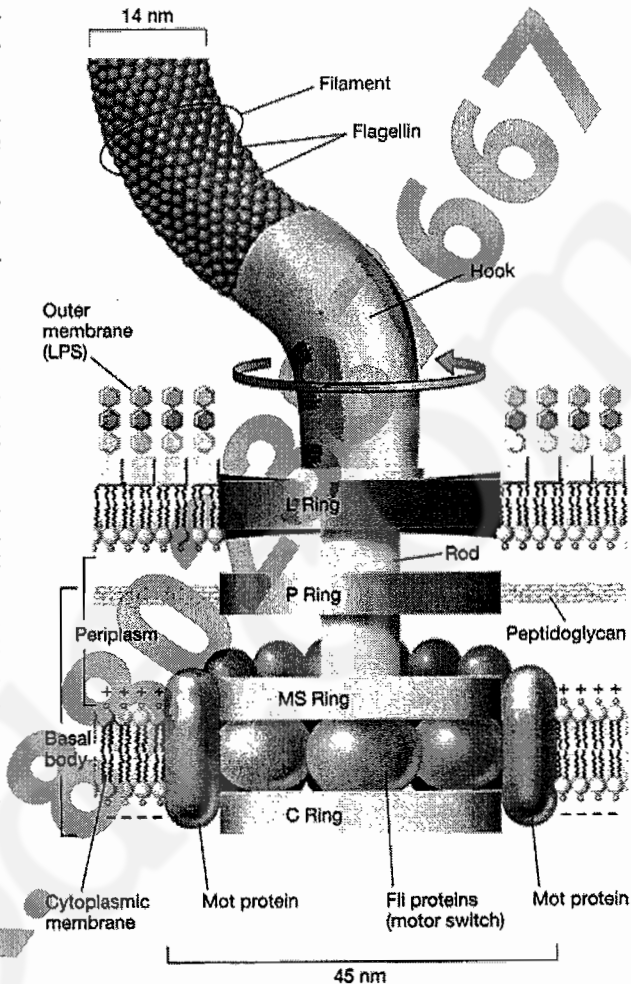


Figure: The molecular organization of bacterial flagella

Pili

The terms pili and fimbriae are usually used interchangeably to describe the thin, hairlike appendages on the surface of many Gram-negative bacteria and proteins of pili are referred to as pilins. Pili are more rigid in appearance than flagella. In some organisms, such as *Shigella* species and *E. coli*, pili are distributed profusely over the cell surface, with as many as 200 per cell. As is easily recognized in strains of *E. coli*, pili can come in two types: short, abundant common pili, and a small number (one to six) of very long pili known as sex pili. Sex pili can be distinguished by their ability to bind male-specific bacteriophages (the sex pilus acts as a specific receptor for these bacteriophages). The sex pili attach male to female bacteria during conjugation.

Figure: Various patterns of flagellar insertions in bacterial cells.

Structure	Flagella Type	Example
	Monotrichous	<i>Vibrio cholerae</i>
	Lophotrichous	<i>Bartonella bacilliformis</i>
	Amphitrichous	<i>Spirillum serpens</i>
	Peritrichous	<i>Escherichia coli</i>

Sex pili are primarily required for conjugation. In Gram positive cells, where there is no sex pilus, conjugation takes place by means of direct cell fusion [Prescott, 2003].

Pili in many enteric bacteria

confer adhesive properties on the bacterial cells, enabling them to adhere to various epithelial surfaces, to red blood cells (causing hemagglutination), and to surfaces of yeast and fungal cells. These adhesive properties of piliated cells play an important role in bacterial colonization of epithelial surfaces and are therefore referred to as **colonization factors**.

Cell envelope

Layers from Plasma Membrane outwards are referred to as the cell envelope. The cytoplasm of all bacteria is enclosed within a plasma (cytoplasmic) membrane external to which, in most cases of eubacteria, is a rigid cell wall made up of sugars and amino acids called peptidoglycan. The role of the cell wall is to protect the cell from lysis resulting from osmotic pressure and it also gives shape to the cell. Some bacteria, the mycoplasma, do not have cell walls and therefore are unable to survive outside an animal host which provides it with the right osmotic environment. Gram-negative bacteria have an additional outer membrane containing LIPOPOLYSACCHARIDES. External to this may be other layers of polysaccharide or protein making up a capsule or slime layer.

The term **bacterial cell envelope** includes four distinct components, which are:

1. Plasma Membrane
2. Cell wall
3. Periplasmic space
4. the Outer membrane

The cell envelope in Gram Positive and Gram Negative Bacteria are different.

1. Plasma Membrane: The plasma membrane is a semi-permeable lipid bilayer with associated proteins, which acts as a barrier between the cytoplasm and the surrounding environment. The membrane is made of phospholipids & sterol analogs such as *hopanoids*.

Proteins of various functions are embedded in the lipid bilayer. These include transport proteins, proteins involved in energy metabolism and receptor proteins that can detect and respond to chemical stimuli. Integral proteins are those that are fully associated with the membrane and may penetrate all the way through. These proteins therefore contain hydrophobic amino acids in the regions which are buried in the lipid. Peripheral proteins are ones that are only loosely associated by charge interactions with the positively charged polar head groups of the phospholipids or on integral proteins and can be removed from purified membranes by washing with salt solutions. The lipids and proteins are fully mobile and move in relation to each other. This widely accepted model of membrane structure is the fluid mosaic model given by Singer & Nicholson.

2. Cell wall: Outside the plasma membrane is the cell wall made of sugars and amino acids called the peptidoglycan (sometimes also called the murein layer). The role of the cell wall is to provide rigidity and strength, preventing the cell from osmotic lysis when placed in dilute environments. The structure of

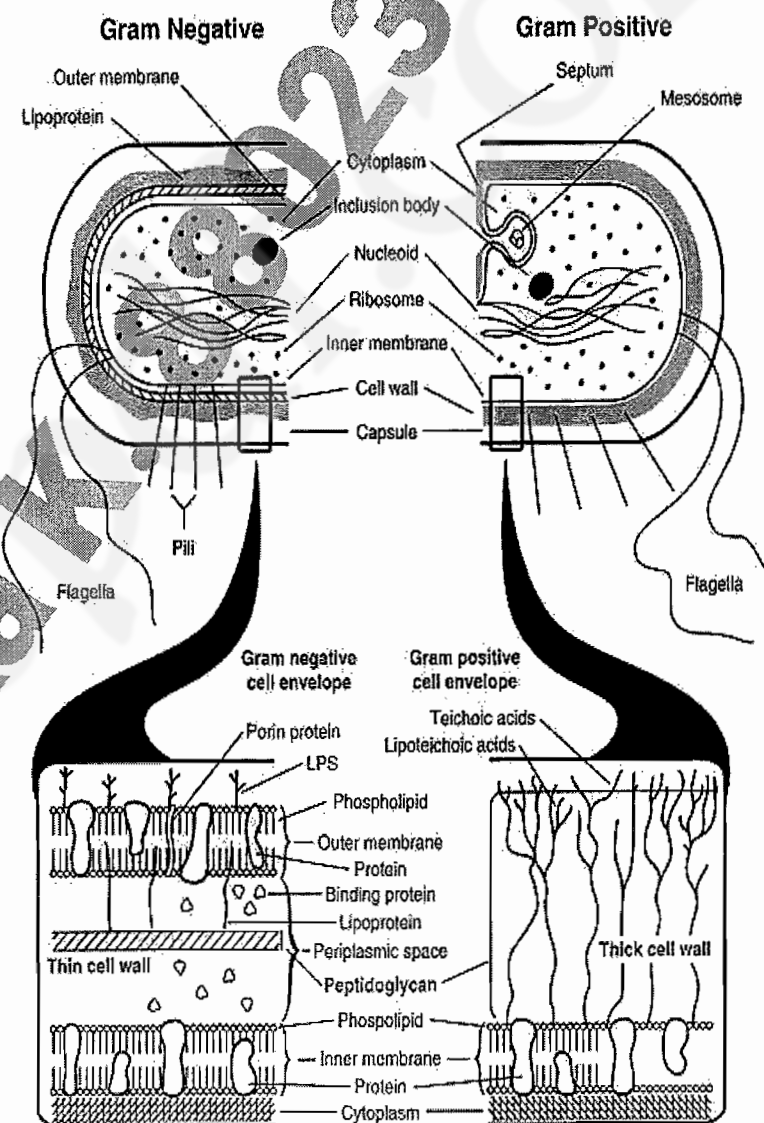


Figure: Cell Envelopes in Gram Positive and Gram Negative Bacteria

The role of the cell wall is to provide rigidity and strength, preventing the cell from osmotic lysis when placed in dilute environments. The structure of

peptidoglycan from *E. coli* has been widely studied. It consists of long polymers of two sugar derivatives, NAG and NAM with side chains of four alternating D- and L-amino acids attached to the NAM. Rigidity is achieved by cross links between the amino acid chains normally from the third amino acid in one chain to the fourth amino acid in another chain. The nature of the amino acid side chains, and the links that join them, vary between bacterial species.

Multiple cross links within and between chains makes peptidoglycan a very strong and rigid structure. There are several unique features of peptidoglycan including: the presence of NAM; this sugar is not found in eukaryotic cells; & the presence of D-amino acids; L amino acids are normally found in proteins. These features make the peptidoglycan a target for antimicrobial agents that destroy prokaryotic cells' specifically, but do not harm eukaryotic cells; an example of this is the antibiotic penicillin.

Lysozyme, a natural antibacterial agent found in tears and natural secretions breaks down the β (1-4) linkage between NAM and NAG. Removal of the cell wall under conditions where the osmolarity of the medium is the same as the inside of the cell (isotonic solution) results in the formation of round protoplasts (Gram positives) or spheroplasts (Gram-negatives) which survive as long as the isotonicity is maintained. These structures lyse, however, if placed in a dilute medium, illustrating the importance of peptidoglycan to the cells' survival.

Gram-positive cell walls also contain large amounts of another polymer, called **teichoic acid**, made up of glycerol or ribitol joined by phosphate groups. Cell walls of a number of genera including *Mycobacterium*, *Corynebacteria* and *Nocardia* contain waxy esters of mycolic acids, which are complex fatty acids.

3. Periplasmic space: The outer membrane of Gram-negative bacteria acts as an additional barrier protecting the peptidoglycan from toxic compounds such as lysozyme which act on the cell wall. It creates an aqueous space between the two membranes called the periplasmic space which is thought to have a gel like structure with a loose network of peptidoglycan running through it. The periplasmic space contains a range of proteins associated with:

- transport of nutrients into the cell
- enzymes that are involved in nutrient acquisition such as proteases
- enzymes that defend the cell against toxic chemicals such as β -lactamases that destroy penicillin

In Gram-positive cells these enzymes (called exoenzymes) are normally secreted into the surrounding medium. The presence of the outer membrane in Gram-negative bacteria allows the cell to keep the enzymes close to itself rather than losing them into the medium.

4. The outer membrane: The outer membrane of Gram-negative bacteria is made up of phospholipids but it also contains some unique features.

- Pores formed by proteins called porins such as OmpF and OmpC that allow the passive diffusion of small molecules into the periplasmic space.
- An abundant small lipoprotein called Braun's lipoprotein that is covalently bound to the peptidoglycan and is embedded in the outer membrane by its hydrophobic lipid, therefore holding the peptidoglycan and outer membrane close together.
- Lipopolysaccharide [LPS] molecules are found in the outer leaflet of the outer membrane projecting into the surrounding medium.

LPS is responsible for a number of features of the Gram-negative bacterium:

- they cause a net negative charge on the surface of the cell
- they may hinder the access of toxic molecules to the surface of the cell & therefore play a protective role
- the long side chains are capable of variation in structure, therefore may play a role in allowing Gram-negative bacteria to evade the immune response

Most importantly, the lipid A portion of the LPS molecule is highly toxic to mammals. Called endotoxin, its presence in the blood stream, even at very low concentrations, leads to toxic shock and death.

Different words are used to describe the layers of material often seen on the surface of cells. A well organized dense structure is called a capsule. If it is diffuse and easily lost it is called a slime layer. Glycocalyx is often used to describe both capsules and slime layers that are made up of polysaccharides; however, some bacteria have proteinaceous capsules.

These additional surface layers have a number of functions:

- they act as permeability barriers to the cell surface;
- they protect the bacteria from phagocytosis;
- they protect the cell from desiccation;
- they aid in the attachment (adhesion) of bacteria to surfaces

Cytoplasmic components

The cytoplasm of bacteria is aqueous, containing a number of molecules, ribonucleic acid (RNA) and proteins necessary for the cell functions. The main structures, common to all bacteria, found in the cytoplasm are the ribosomes, which are of 70S type, both in Archaea & Eubacteria. The ribosomes are the sites of protein synthesis in the cell. The ribosomes in prokaryotic cells, although similar in shape and function to those of eukaryotic cells, are different in the nature of the proteins and RNAs that make up their structure. This has proved to be very useful to the human population as antibiotics that act by inhibiting protein synthesis in the bacteria are not effective against eukaryotic protein synthesis, thus allowing selective toxicity.

Some bacteria contain structures associated with specialized functions.

1. Granular structures, called inclusion bodies, can often be seen under the light microscope. These granules are usually for storage and may be membrane bound, such as poly- β -hydroxybutyrate (PHB) granules, or found scattered in the cytoplasm, such as polyphosphate granules (also called metachromatic granules). Lipid droplets can also be seen in some bacteria. An interesting inclusion body, the gas vacuole, is found in Cyanobacteria (blue-green algae) and other photosynthetic bacteria that live in an aqueous environment. This protein-surrounded vacuole provides buoyancy, allowing the bacteria to float near the surface of the water.
2. Although bacteria do not have organized intracellular membranes, invaginations of the plasma membrane called mesosomes are often seen in electron micrographs. There is some debate as to whether the mesosomes really exist and are not just an artefact of the fixation process for electron microscopy. Their function is thought to be in cell division, possibly in laying down new cell-wall material or in the replication of the chromosome and its subsequent distribution to daughter cells.
3. More specialized and complex, intracellular membrane systems are found in photosynthetic bacteria, such as Chloroflexus (green non-sulfur bacteria) and Rhodospirillum rubrum (purple bacterium), associated with the trapping of light energy.
4. Magnetosome: The magnetosome chains are membranous prokaryotic organelles present in magnetotactic bacteria. They contain 15 to 20 magnetite crystals that together act like the needle of a compass to orient magnetotactic bacteria in geomagnetic fields, thereby simplifying their search for their preferred microaerophilic environments. Each magnetite crystal within a magnetosome is surrounded by a lipid bilayer, and specific soluble and transmembrane proteins are sorted to the membrane. Magnetotactic bacteria usually mineralize either iron oxide magnetosomes, which contain crystals of magnetite (Fe_3O_4), or iron sulphide magnetosomes, which contain crystals of greigite (Fe_3S_4). Several other iron sulphide minerals have also been identified in iron sulphide magnetosomes — including mackinawite (tetragonal FeS) and a cubic FeS — which are thought to be precursors of Fe_3S_4 . Recent research has shown that magnetosomes are invaginations of the inner membrane and not freestanding vesicles. Magnetite-bearing magnetosomes have also been found in eukaryotic magnetotactic algae, with each cell containing several thousand crystals.

Bacterial DNA

It is located within the cytoplasm. Although the chromosome is not contained within a nuclear membrane, it is often seen as a discrete area within the cell in electron micrographs which may be referred to as the nucleoid.

It consists of a single chromosome which varies in size between different species of bacteria (the *E. coli* chromosome is 4×10^6 base pairs long). The DNA is circular and tightly super coiled, but *Borrelia burgdorferi* and *Streptomyces coelicolor* have linear dsDNA.

The DNA is associated with proteins which are similar to the histone proteins [but not true Histones found in eukaryotic cells]. The complexing proteins binding to DNA are 3 in total:

1. HLP: small molecular weight, bind to DNA through minor grooves at regular intervals
2. HU: a dimeric protein (with hua and hub units) that binds to DNA and condenses it in bead like structure; also known to stimulate DNA replication.
3. H1: also called H-NS, preferentially binds to bend DNA regions

Some bacteria also contain small molecules of extra-chromosomal DNA called plasmids. These often carry genes which are not essential to the normal life of the cell but confer an advantage to the cell in certain situations such as antibiotic-resistant plasmids. The chromosome in archaea, like that of the eubacteria, is a single, circular DNA molecule not contained within a nucleus, but the size of the DNA molecule is often smaller than that of Eubacteria.

Gram Staining

Gram staining (or Gram's Method) is an empirical method of differentiating bacterial species into two large groups based on the chemical and physical properties of their cell walls. In the process of making diagnosis of infectious diseases, bacteriological tests must be done. The Gram staining represents one of such tests.

It is a differential staining process developed by Danish physician **Häns Christian Gram** in 1884.

The eubacteria are frequently divided into two groups – Gram positive and Gram negative – on the basis of their differential reaction to the Gram Staining. (A few organisms are consistently Gram-variable.)

Step	Gram-positive organisms	Gram-negative organisms
1. Unstained	Clear	Clear
2. Crystal violet	Violet	Violet
3. Iodine	Violet	Violet
4. Decolorization (alcohol-acetone)	Violet	Clear
5. Safranin	Purple	Red

The procedure

The procedure for the Gram stain is as follows.

1. The first step is the correct preparation of the smear- which is a thin film of the material on a clean glass slide. The cells are fixed then.
2. Fixed cells are stained with a dark stain such as crystal violet. To do so, slide is flooded with crystal violet for 10 seconds. After which we wash it with running tap water or a maximum of 5 seconds.
3. Iodine (Gram's iodine) solution is added (1% iodine, 2% potassium iodide in water) for 1 minute. This acts as a mordant and fixes the dye in the cell wall. It is rinsed with water.
4. 95% ethanol or a mixture of acetone and alcohol is applied several times until no more colour appears to come from the sample. This washes away all the unbound stain and leaves Gram-positive organisms stained purple and Gram-negative organisms unstained (colourless).
5. Finally, a counter-stain such as carbol fuchsin or safranin is applied, which stains the decolorized cells pink. However, the counter stain is not seen on the dark staining cells which retained the first stain.

Interpretation: The cells that retain the stain (with thick cell walls) are called Gram-positive and appear dark purple under light microscopy. The ones that lose the stain (with thin cell walls and an outer membrane) are called Gram-negative.

The basis

The differential reaction to the staining procedure is because of the structure of the cell envelope in these two groups of bacteria.

Gram-positive bacteria have a thicker peptidoglycan cell wall, which retains the violet dye/iodine complex. Gram-negative bacteria have a thin cell wall, which is covered by an outer lipid membrane. As a result of this lipid membrane and thin wall, the gram negative cells take up much lesser amounts of the stain. Most of the stain is stopped at the outer membrane only – which gets washed by the decolorising mixture. The same decolorising mixture causes dehydration of the multilayered peptidoglycan in the Gram-positive cell wall (due to presence of teichoic acid), thus trapping the crystal violet-iodine complex within the cell.

Significance

Research: Gram staining is one of the most useful staining procedures in bacteriological laboratory. The technique is widely used as a tool for differentiating and identifying a particular bacterial sample.

Medical: Gram stains are performed on body fluid or biopsy when infection is suspected. It yields results much quicker than culture, and is especially important when infection would make an important difference in the patient's treatment and prognosis; examples are cerebrospinal fluid for meningitis and synovial fluid for septic arthritis. Gram-negative bacteria are more dangerous as disease organisms, because:

- Their outer membrane is often hidden by a capsule layer which hides the antigens and prevents phagocytosis
- Lipopolysaccharide in their outer membrane acts as an endotoxin which increases the severity of inflammation. This inflammation may be so severe that septic shock may occur.

- They often contain antibiotic breaking enzymes such as beta-lactamases (which break beta-lactam antibiotics, such as penicillin), which may hinder the access of toxic molecules to the surface of the cell and therefore play a protective role.
- The long side chains of polysaccharides present in the outer membrane are capable of variation in structure, therefore may play a role in allowing Gram-negative bacteria to evade the immune response

Gram-positive infections are generally less severe because the human body produces an enzyme called lysozyme which attacks the open peptidoglycan layer of Gram-positive bacteria. Gram-positive bacteria are also frequently much more susceptible to beta-lactam antibiotics, such as penicillin.

Summary of difference between Gram Positive and Gram Negative Bacteria

Gram-positive and Gram-negative organisms differ drastically in the organization of the cell envelope. Difference in cell envelope organization provides the basis of Gram Staining.

Most Gram-positive bacteria have a relatively thick (about 20 to 80 nm), continuous cell wall (often called the sacculus), which is composed largely of peptidoglycan (also known as mucopeptide or murein). In thick cell walls, other cell wall polymers (such as the teichoic acids, polysaccharides, and peptidoglycolipids) are covalently attached to the peptidoglycan.

In contrast, the peptidoglycan layer in Gram-negative bacteria is thin (about 5 to 10 nm thick); in *E. coli*, the peptidoglycan is probably only a monolayer thick. Outside the peptidoglycan layer in the Gram-negative envelope is an outer membrane structure (about 7.5 to 10 nm thick). In most Gram-negative bacteria, this membrane structure is anchored noncovalently to lipoprotein molecules (Braun's lipoprotein), which, in turn, are covalently linked to the peptidoglycan.

Apart from cell envelope organization, the two classes of bacteria also differ in:

1. mesosomes being better developed in gram positive cells
2. pili being better developed in gram negative cells
3. conjugation being more frequent in gram negative cells

Bacterial multiplication

Most bacteria rely on **binary fission** for propagation. This is a simple process; a cell grows to twice its starting size and then split in two. Before binary fission occurs, the cell must copy its genetic material (DNA) and segregate these copies to opposite ends of the cell.

Then the many types of proteins that comprise the cell division machinery assemble at the future division site.

A key component of this machinery is the **protein FtsZ**. Protein monomers of FtsZ assemble into a ring-like structure at the center of a cell. Other components of the division apparatus then assemble at the FtsZ ring. This machinery is positioned so that division splits the cytoplasm and does not damage DNA in the process. As division occurs, the cytoplasm is cleaved in two, and in many bacteria, new cell wall is synthesized. The order and timing of these processes (DNA replication, DNA segregation, division site selection, invagination of the cell envelope and synthesis of new cell wall) are tightly controlled.

BINARY FISSION:

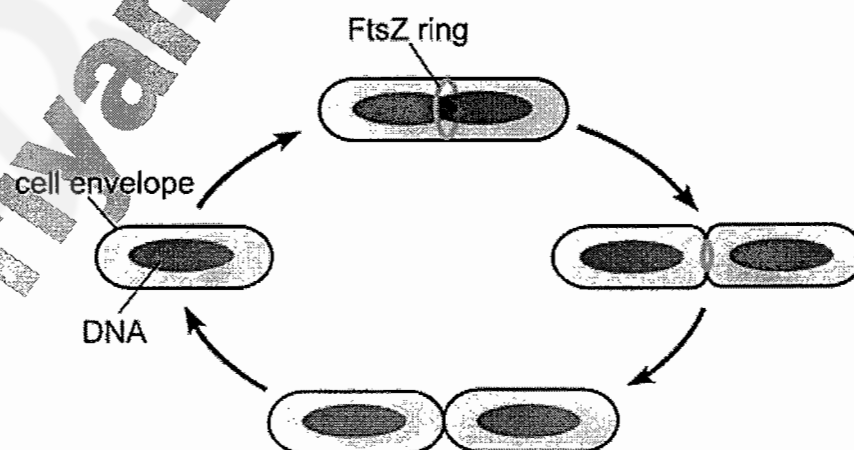


Figure: Bacterial Binary Fission

Some unusual forms of reproduction in bacteria

Baeocyte production in the cyanobacterium *Stanieria*

Stanieria never undergoes binary fission. It starts out as a small, spherical cell approximately 1 to 2 µm in diameter. This cell is referred to as a baeocyte (which literally means 'small cell'). The baeocyte begins to grow, eventually forming a vegetative cell up to 30 µm in diameter. As it grows, the cellular DNA is replicated over and over, and the cell produces a thick extracellular matrix. The vegetative cell eventually transitions into a reproductive phase where it undergoes a rapid succession of cytoplasmic fissions to produce dozens or even hundreds of baeocytes. The extracellular matrix eventually tears open, releasing the baeocytes.

Bacterial budding

Budding has been observed in some members of the Planctomycetes, Cyanobacteria, Firmicutes (the Low G+C Gram-Positive Bacteria) and the prosthecate Proteobacteria. Although budding has been extensively studied in the eukaryotic yeast *Saccharomyces cerevisiae*, the molecular mechanisms of bud formation in bacteria are not known.

Chapter 7: Bacterial genetic transfer

Introduction to bacterial genetic exchange

The bacteria are organisms without sexual reproduction, however they carry out genetic exchange by *parasexual modes*.

This process of genetic exchange in bacteria is *unique* because it brings about horizontal genetic transfer. It is usually not seen in eukaryotes.

The additional points of distinction between bacterial and eukaryotic genetic exchange are:

1. DNA exchange and reproduction are not coupled in bacteria.
2. Gene transfer in bacteria is *unidirectional* from a donor cell to a recipient cell and the donor usually gives only a small part of its DNA to the recipient. Thus, complete zygotes are not formed; rather, partial zygotes (*merozygotes*) are formed.
3. Donated genetic material that is not recombined into the host DNA is usually degraded, and so the recipient cell remains haploid.

Bacterial plasmids

Many bacteria possess plasmids in addition to their chromosome. These are double-stranded DNA molecules, usually circular, that can exist and replicate independently of the chromosome or may be integrated with it; in either case they normally are inherited or passed on to the progeny. However, plasmids are not usually attached to the plasma membrane and sometimes are lost to one of the progeny cells during division.

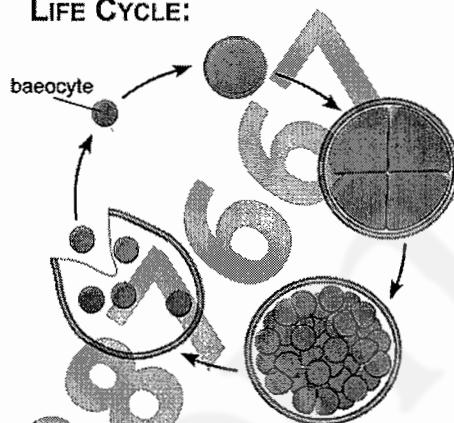
Plasmids are not required for host growth and reproduction, although they may carry genes that give their bacterial host a selective advantage. Plasmid genes can render bacteria drug-resistant, give them new metabolic abilities, make them pathogenic, or endow them with a number of other properties. Because plasmids often move between bacteria, properties such as drug resistance can spread throughout a population.

Structure and properties

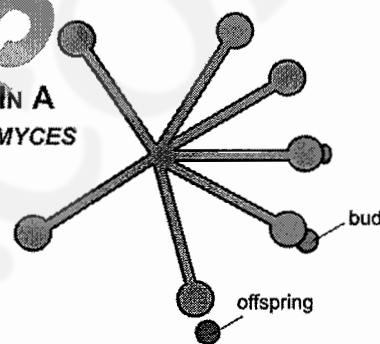
Plasmids are small double-stranded DNA molecules, usually circular, that can exist independently of host chromosomes and are present in many bacteria (they are also present in some yeasts and other fungi).

They have their own replication origins and are autonomously replicating and stably inherited. A replicon is a DNA molecule or sequence that has a replication origin and is capable of being replicated. Plasmids and bacterial chromosomes are separate replicons.

THE *STANIERIA*
LIFE CYCLE:



BUDDING IN A
PLANCTOMYCES
SPECIES:



Plasmids have relatively few genes, generally less than 30. Their genetic information is not essential to the host, and bacteria that lack them usually function normally. Single-copy plasmids produce only one copy per host cell. Multicopy plasmids may be present at concentrations of 40 or more per cell.

Characteristically, plasmids can be eliminated from host cells in a process known as **curing**. Curing may occur spontaneously or be induced by treatments that inhibit plasmid replication while not affecting host cell reproduction. Some commonly used curing treatments are acridine mutagens, UV and ionizing radiation, thymine starvation, and growth above optimal temperatures.

Types

Plasmids may be classified in terms of their mode of existence and spread.

Based on function, there are five main classes:

1. **Fertility F-plasmids**, which contain *tra* genes. They are capable of conjugation and result in the expression of sex pili.
2. **Resistance plasmids**, which contain genes that provide resistance against antibiotics or poisons. They were historically known as R-factors, before the nature of plasmids was understood.
3. **Col plasmids**, which contain genes that code for bacteriocins, proteins that can kill other bacteria.
4. **Virulence plasmids**, which turn the bacterium into a pathogen.
5. **Degradative plasmids**, also called **metabolic plasmids** which enable the digestion of unusual substances, e.g. toluene and salicylic acid.

A brief summary of the types of plasmids and their properties is given in Table 1.

Type	Representatives	Approximate Size (kbp)	Copy Number (Copies/Chromosome)	Hosts	Phenotypic Features*
Fertility Factor^b	F factor	95–100	1–3	<i>E. coli</i> , <i>Salmonella</i> , <i>Citrobacter</i>	Sex pilus, conjugation
R Plasmids	RP4	54	1–3	<i>Pseudomonas</i> and many other gram-negative bacteria	Sex pilus, conjugation, resistance to Ap, Km, Nm, Tc
	R1	80	1–3	Gram-negative bacteria	Resistance to Ap, Km, Su, Cm, Sm
	R6	98	1–3	<i>E. coli</i> , <i>Proteus mirabilis</i>	Su, Sm, Cm, Tc, Km, Nm
	R100	90	1–3	<i>E. coli</i> , <i>Shigella</i> , <i>Salmonella</i> , <i>Proteus</i>	Cm, Sm, Su, Tc, Hg
	pSH6	21		<i>Staphylococcus aureus</i>	Gm, Tm, Km
	pSJ23a	36		<i>S. aureus</i>	Pn, Asa, Hg, Gm, Km, Nm, Em, etc.
	pAD2	25		<i>Enterococcus faecalis</i>	Em, Km, Sm
Col Plasmids	ColE1	9	10–30	<i>E. coli</i>	Colicin E1 production
	ColE2		10–15	<i>Shigella</i>	Colicin E2
	ColDF13			<i>Enterobacter cloacae</i>	Cloacin DF13
Virulence Plasmids	Ent (P307)	83		<i>E. coli</i>	Enterotoxin production
	K88 plasmid			<i>E. coli</i>	Adherence antigens
	ColV-K30	2		<i>E. coli</i>	Siderophore for iron uptake; resistance to immune mechanisms
	pZA10	56		<i>S. aureus</i>	Enterotoxin B
	Ti	200		<i>Agrobacterium tumefaciens</i>	Tumor induction
Metabolic Plasmids	CAM	230		<i>Pseudomonas</i>	Camphor degradation
	SAL	56		<i>Pseudomonas</i>	Salicylate degradation
	TOL	75		<i>Pseudomonas putida</i>	Toluene degradation
	pJP4			<i>Pseudomonas</i>	2,4-dichlorophenoxyacetic acid degradation
				<i>E. coli</i> , <i>Klebsiella</i> , <i>Salmonella</i>	Lactose degradation
				<i>Providencia</i>	Urease
	sym			<i>Rhizobium</i>	Nitrogen fixation and symbiosis

*Abbreviations used for resistance to antibiotics and metals: Ap, ampicillin; Asa, arsenate; Cm, chloramphenicol; Em, erythromycin; Gm, gentamicin; Hg, mercury; Km, kanamycin; Nm, neomycin; Pn, penicillin; Su, streptomycin; So, sulfonamide; Tc, tetracycline.

^bMany R plasmids, metabolic plasmids, and others are also conjugative.

Modes of bacterial genetic exchange

There are three main modes of bacterial genetic exchange. None of these is true sexual reproduction.

1. Conjugation
2. Transformation
3. Transduction

Conjugation

Conjugation is the passage of genetic material – mostly a plasmid – directly from one bacterium to another through a tubular connection between the two cells. This tubular connection is the *conjugation tube* in gram negative bacteria. Most gram positive bacteria do not make conjugation tubes but they conjugate by mechanism of localized cellular fusions.

Conjugation was discovered by Joshua Lederberg and Edward Tatum in 1946; however the mechanism was explained in detail by Bernard Davis in 1956.

The process of conjugation

In conjugation, two bacteria lie close together and a connection forms between them. A plasmid or a part of the bacterial chromosome passes from one cell (the donor) to the other (the recipient). After conjugation, crossing over may occur between homologous sequences in the transferred DNA and the chromosome of the recipient cell. In conjugation, DNA is transferred only from donor to recipient and there is no reciprocal exchange of genetic material.

The role of F-factor in conjugation

In most bacteria, conjugation depends on a fertility (F) factor or F plasmid or Sexmid. It is present in the donor cell and absent in the recipient cell. Cells that contain F are referred to as F⁺, and cells lacking F are F⁻.

The F factor contains an origin of replication and a number of genes required for conjugation within a region called *tra* (transfer) region. A cell containing F produces the sex pili, one of which makes contact with a receptor on an F⁻ cell and pulls the two cells together. DNA is then transferred from the F⁺ cell to the F⁻ cell.

DNA Transfer is initiated when one of the DNA strands on the F factor is nicked at an *Origin of Transfer* location (*oriT*). One end of the nicked DNA separates from the circle and passes into the recipient cell. The donor DNA is replicated by *rolling circle mechanism*. It thus become double stranded again by the time one strand has been successfully transferred to the recipient cell. This mechanism is explained in figure 1.

Inside the recipient cell, the single strand is replicated producing a circular, double-stranded copy of the F plasmid. This step is called *Replication*. If the entire F factor is successfully transferred to the recipient F⁻ cell, that cell becomes an F⁺ cell.

Formation of Hfr Strains: The F-factor is an episome. It can integrate into the bacterial chromosome or can stay independently in the bacterial cell. When the F factor is integrated into the bacterial chromosome, the host cell is called Hfr (Figure 2). Hfr cells behave as F⁺ cells, forming sex pili and undergoing conjugation with F⁻ cells.

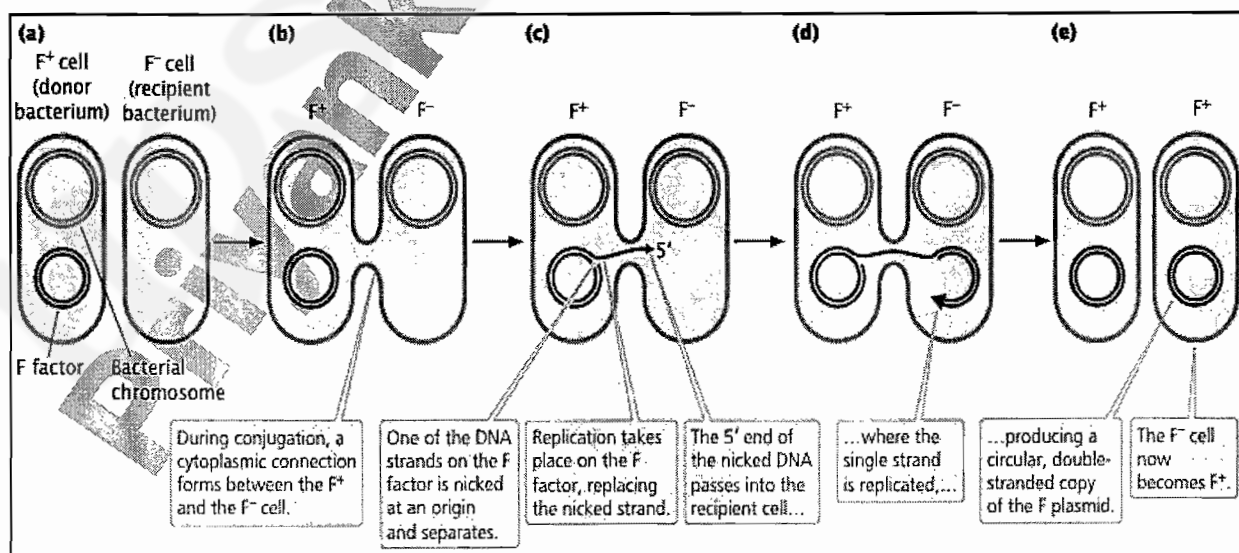


Figure 1: The process of conjugation between F⁺ cell and F⁻ cell

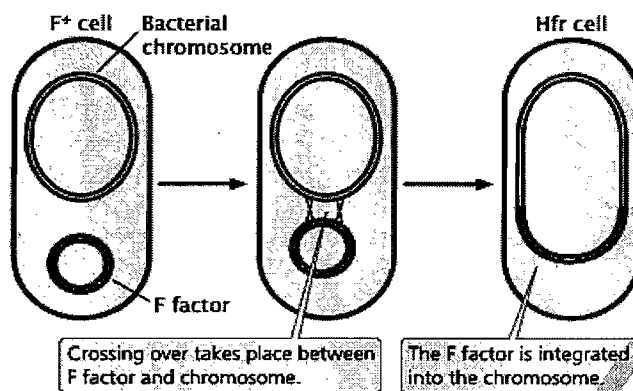


Figure 2: The formation of Hfr cell

In conjugation between Hfr and F⁻ cells the host chromosome with the integrated F factor is nicked in the region *OriT*. The the end of the nicked strand moves into the F⁻ cell. In the Hfr cells, as the F factor is linked to the bacterial chromosome, the chromosome also starts passing into the recipient cell.

However, in a mating of Hfr and F⁻, the F⁻ cell almost never becomes F⁺ or Hfr, because the F factor is nicked in the middle during the initiation of strand transfer, placing a part of F at the beginning and part at the end of the strand to be transferred. To become F⁺ or Hfr, the recipient cell must receive the entire F factor, requiring that the entire bacterial chromosome is transferred. This event mostly does not happen, because conjugating cells break apart before the entire chromosome has been transferred.

Formation of F' plasmid: When an F factor excises from the bacterial chromosome, a small amount of the bacterial chromosome may be removed with it, and these chromosomal genes will then be carried with the F plasmid. Cells containing an F plasmid with some bacterial genes are called *F prime* (F'). They act as Donors.

The mechanism of conjugation in Gram + bacteria is different than that for Gram -. In Gram + bacteria the donor makes an adhesive material which causes aggregation and localized fusion with the recipient and the DNA is transferred.

Importance of conjugation

1. Among the Gram negative bacteria this is the major way that bacterial genes are transferred.
2. Transfer can occur between different species of bacteria. Transfer of multiple antibiotic resistance by conjugation has become a major problem in the treatment of certain bacterial diseases. Since the recipient cell becomes a donor after transfer of a plasmid, therefore the spread of resistance is very fast.
3. Gram positive bacteria also have plasmids that carry multiple antibiotic resistance genes, in some cases these plasmids are transferred by conjugation.

Transformation

Transformation is a process by which a bacterium takes up DNA from the surrounding medium and then recombination occurs between the introduced DNA and the bacterial chromosome.

The first demonstration of bacterial transformation was done with *Streptococcus pneumoniae* in 1928 by Frederick Griffith. It also led to the discovery that DNA is the genetic material.

Occurrence

Certain bacteria carry out transformation with greater frequency; they are *Bacillus*, *Streptococcus*, *Thermoactinomyces*, *Azotobacter*, *Helicobacter*, *Pseudomonas*, *Haemophilus*, *Neisseria*, *Pneumococcus* and some related genera.

Process

Transformation takes place to a limited extent in some conditions. Now, some laboratory techniques have also been developed to increase the rate of DNA uptake. These techniques, such as CaCl₂ treatment or Electroporation, are widely used in Recombinant DNA Technology.

Factors affecting transformation are:

1. **DNA size state** – Double stranded DNA of at least 5 X 10⁵ daltons works best. Thus, transformation is sensitive to nucleases in the environment.
2. **Competence of the recipient** – Some bacteria are able to take up DNA naturally but only during a particular time in their growth cycle. This is known as the competency phase of the bacterium. In

this phase the bacteria produce a specific protein called a *competence factor*. This factor stimulates the formation of other proteins related to transformation.

Steps in transformation are:

1. **Uptake of DNA** – Uptake of DNA by Gram+ and Gram- bacteria differs. In Gram + bacteria the DNA is taken up as a single stranded molecule and the complementary strand is made in the recipient. In contrast, Gram- bacteria take up double stranded DNA.

There are certain proteins present in the cell wall and cell membrane which help in the uptake of DNA from outside. Some of these important proteins are:

PilQ; ComE; Com A; Com EC and ComFA.

2. **General Recombination** – After the donor DNA is taken up, a recombination event occurs between the chromosome and the donor DNA. This recombination requires homology between the donor DNA and the chromosome. It results in the substitution of DNA between the recipient and the donor. Recombination requires the bacterial recombination proteins (*recBCD* complex and *recA*) and homology between the DNA's involved. This type of recombination is called *homologous* or *general recombination*. Because of the requirement for homology between the donor and host DNA, only DNA from closely related bacteria can successfully transform. But, in rare instances gene transfer between distantly related bacteria has been shown to occur.

Significance

Transformation occurs in nature and it can lead to increased virulence. In addition transformation is widely used in recombinant DNA technology.

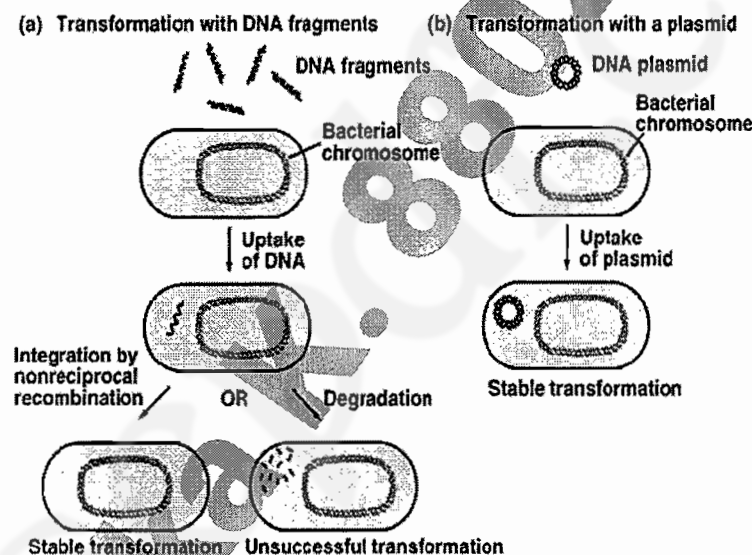


Figure 3: Bacterial Transformation

Transduction

Transduction is the transfer of genetic information from a donor to a recipient bacterium by way of a bacteriophage.

Transduction takes place when bacterial viruses carry small segments of the previous host cell's DNA to the next host cell. Inside the bacterium, the newly introduced DNA may undergo recombination with the bacterial chromosome. If it does not, it will be destroyed.

Occurrence

Not all phages can mediate transduction. The ability of a phage to mediate transduction is related to the life cycle of the phage. Transduction is always mediated by lysogenic viruses after lytic induction.

Most bacteriophages have a limited host range; so transduction is normally between bacteria of the same or closely related species only. However, if a particular phage has a wide host range then transfer between widely separated species can also occur.

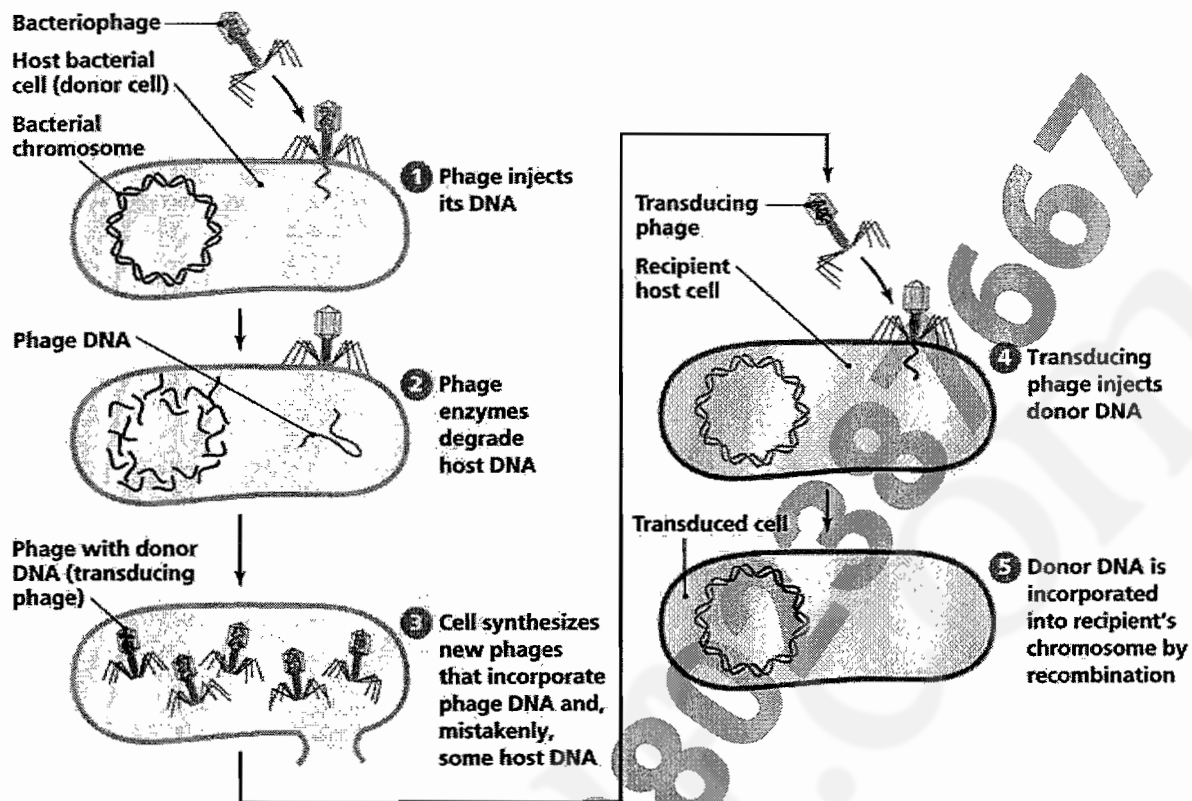


Figure 4: Bacterial transduction

Types of Transduction

There are two types of transduction

1. **Generalized Transduction** is transduction in which potentially any bacterial gene from the donor can be transferred to the recipient. Phages that mediate generalized transduction generally breakdown host DNA into smaller pieces. Occasionally one of the pieces of host DNA is randomly packaged into a phage coat. Thus, any donor gene can be potentially transferred. When a recipient cell is infected by a phage that contains donor DNA, donor DNA enters the recipient. In the recipient a generalized recombination event can occur which substitutes the donor DNA and recipient DNA.
2. **Specialized transduction** is transduction in which only certain donor genes can be transferred to the recipient. Specialized transduction is mediated by lysogenic or temperate phage and the genes that get transferred are the flanking genes from the location where the prophage has inserted in the chromosome.

During excision of the prophage, occasionally an error occurs where some of the host DNA is excised with the phage DNA. Only host DNA on either side of where the prophage has inserted can be transferred. It is therefore called specialized transduction. After replication and release of phage and infection of a recipient, lysogenization of recipient can occur resulting in the stable transfer of donor genes.

Significance

1. Lysogenic (phage) conversion occurs in nature and is the source of virulent strains of bacteria.
2. The phage coat protects the DNA in the environment so that transduction, unlike transformation, is not affected by nucleases in the environment.

Mycoplasmas

An introduction to mycoplasmas

Mycoplasmas are spherical to filamentous prokaryotic cells with no cell walls. Due to the absence of cell wall, they are also called Mollicutes (= soft skinned). Mycoplasmas are the second smallest known self-replicating organisms (size ranging from 500nm to 5µm) with the smallest genomes (a total of about 500 to 1000 genes) – after *Nanoarchaeum equitans* (an Archaea; diameter: ~400 nm). Due to lack of a cell wall, the mycoplasmas do not give response to Gram Staining but the mycoplasmas presumably evolved by degenerative evolution from non-sporulating, non-endospore forming Gram-positive bacteria. Based on 16S rRNA comparisons, they are found phylogenetically most closely related to some Clostridia.

The following eight genera of the mycoplasmas with a total of about 130 recognized species are most widespread among the mollicutes.

1. *Mycoplasma*
2. *Anaeroplasm*
3. *Spiroplasma*
4. *Ureaplasma*
5. *Entomoplasm*
6. *Acholeplasma*
7. *Asteroleplasma*
8. *Mesoplasm*

Taxonomic position of the mycoplasmas

Volume 3 of Bergey's Manual surveys the gram-positive bacteria with low G-C content in their DNA, which are members of the phylum **Firmicutes**. The dividing line is about 50% G-C; bacteria with a mol% lower than this value are in volume 3. Most of these bacteria are gram positive and heterotrophic. However, because of their close relationship to low G-C gram-positive bacteria, the mycoplasmas are placed here even though they lack cell walls and stain gram negative.

The phylum contains three classes.

1. **Class I—Clostridia**. This class contains three orders and 11 families. Although they vary in morphology and size, the members tend to be anaerobic. Genera such as *Clostridium*, *Desulfotomaculum*, and *Sporohalobacter* form true bacterial endospores; many others do not. *Clostridium* is one of the largest bacterial genera.
2. **Class II—Mollicutes**. The class Mollicutes contains five orders and six families. *Members of the class often are called mycoplasmas*. These bacteria lack cell walls and cannot make peptidoglycan or its precursors. Because mycoplasmas are bounded by the plasma membrane, they are pleomorphic and vary in shape from cocci to helical or branched filaments. They are normally nonmotile and stain gram negative because of the absence of a cell wall. In contrast with almost all other bacteria, most species require sterols for growth. The genera *Mycoplasma* and *Spiroplasma* contain several important animal and plant pathogens.
3. **Class III—Bacilli**. This large class comprises a wide variety of gram positive, aerobic or facultatively anaerobic, rods and cocci. The class Bacilli has two orders, Bacillales and Lactobacillales, and 16 families. As with the members of the class Clostridia, some genera (e.g., *Bacillus*, *Sporosarcina*, *Paenibacillus*, and *Sporolactobacillus*) form true endospores.

Diversity of the Mycoplasmas

The class Mollicutes has five orders and six families.

The best-studied genera are found in the orders **Mycoplasmatales** (*Mycoplasma*, *Ureaplasma*), **Entomoplasmatales** (*Entomoplasma*, *Mesoplasma*, *Spiroplasma*), **Acholeplasmatales** (*Acholeplasma*), and **Anaeroplasmatales** (*Anaeroplasma*, *Asteroleplasma*).

The properties of the mycoplasmas

The properties of the mycoplasmas are as follows:

1. Absence of cell wall, which is readily observable with an electron microscope or in chemical analysis (showing lack of DAP, NAM etc). These bacteria lack cell walls because cannot synthesize peptidoglycan precursors.
2. No sensitivity towards Penicillin, sulphonamides and Vancomycin, which act by inhibiting cell wall synthesis in bacteria.
3. Because they are bounded only by a plasma membrane, these prokaryotes are pleomorphic and vary in shape from spherical or pear-shaped organisms to branched or helical filaments. Some mycoplasmas (e.g., *M. genitalium*) have a specialized terminal structure that projects from the cell and gives them a flask or pear shape. This structure aids in attachment to eucaryotic cells.
4. They are osmotically labile due to absence of cell wall but more stable than ordinary protoplasts due to greater abundance of sterols and lipoglycans (long chain heteropolysaccharides linked to membrane lipids) in the plasma membrane. The mycoplasmas with less sterol in the plasma membrane require an osmotically protected medium or sterol in the external medium to survive.
5. When growing on agar, most species will form colonies with a "fried-egg" appearance because they grow into the agar surface at the center while spreading outward on the surface at the colony edges.
6. The presence of small genomes (a total of about 500 to 1000 genes) with low guanine and cytosine content (18–40 mol%). Recently the complete genome of *Mycoplasma genitalium*, a parasite of the human genital and respiratory tracts, has been sequenced. The *M. genitalium* genome is only 580 kilobases long and appears to have 482 genes.
7. Although most are nonmotile, some can glide along liquid-covered surfaces. There is never a flagellum.
8. They usually are facultative anaerobes, but a few are obligate anaerobes.
9. Mycoplasmas can be saprophytes, commensals, or parasites, and many are pathogens of plants, animals, or insects. Mycoplasmas cause several major diseases in livestock, for example, contagious bovine pleuropneumonia in cattle (*M. mycoides*), chronic respiratory disease in chickens (*M. gallisepticum*), and pneumonia in swine (*M. hyopneumoniae*). *M. pneumoniae* causes primary atypical pneumonia in humans, and there is increasing evidence that *M. hominis* and *Ureaplasma urealyticum* also are human pathogens.

Mycoplasmas as plant pathogens

As currently accepted, true mycoplasmas except the *Spiroplasma* sp. are not plant pathogens (G.N. Agrios in *Plant Pathology*, 5th Edition; 2005). However, other wall-less prokaryotes (thus structurally resembling the true mycoplasmas) falling the group, **Phytoplasmas** cause considerable damage as plant pathogens, especially by causing various yellowing diseases.

For many years, the yellows of plants were thought to be caused by viruses. In 1967, however, the Japanese workers, Doi et al discovered pleomorphic mycoplasma like organisms in the phloem cells of plants affected by different yellows type diseases. Currently more than 200 plants diseases are known to be caused by Spiroplasmas and Phytoplasmas.

They occur from temperate to tropical regions but it is in the warmer areas that serious losses occur in crops as coconuts, Citrus, rice, maize, cotton and Potatoes. A number of mollicute diseases of plants are listed by Ghosh and Raychoudhuri (1972).

Pathogenesis by spiroplasmas and phytoplasmas is summarized below.

1. **Spiroplasmas** are helical mollicutes. The spiroplasmas are the only mollicutes showing motility and helical morphology, apparently mediated by a contractile fibrillar cytoskeleton bound to the inner surface of the spiroplasmal membrane. *MreB* and *Spiralin* genes, which are involved in cell-shape determination, have been identified in *S. citri*.

The spiroplasmas are restricted to the phloem sieve tubes and transmitted by phloem sap-feeding insects, as is characteristic of the phytopathogenic mollicutes. So far, Spiroplasmas are known to cause:

- a. Stubborn disease in citrus plants
- b. Brittle root disease in horseradish
- c. Stunt disease in corn plants
- d. A disease with vague symptoms in periwinkle

2. **Phytoplasma**, formerly known as 'Mycoplasma-like organisms' or MLOs, are prokaryotes lacking cell walls that are currently classified in the class *Mollicutes* (Agrios, 1997). Phytoplasmas are associated with plant diseases, and are known to cause more than 200 diseases in several plant species (Kirkpatrick, 1992; McCoy *et al.*, 1989). The genus name *Phytoplasma* is yet to be formally recognised, and is currently at *Candidatus* status, which is used for bacteria that cannot be cultured.

The symptoms shown by infected plants include: yellowing or reddening of the leaves, shortening of the internodes with stunted growth, smaller leaves, excessive proliferation of shoots resulting in a witches' broom, phyllody, virescence, sterile flowers, necrosis of the phloem tissues, dieback of the branches of woody plants, and the general decline and death of the plant.

For many years, the yellows of plants were thought to be caused by viruses. In 1967, however, the Japanese workers, Doi *et al* discovered pleomorphic mycoplasma like organisms in the phloem cells of plants affected by different yellows type diseases.

Phytoplasmas are transmitted from plant to plant by insect vectors, mainly leafhoppers and psyllids (Ploaie, 1981). They traverse the wall of the intestinal tract, multiply in the hemolymph, and pass through the salivary glands, in which they multiply further. Then, the insect vectors introduce phytoplasmas along with salivary fluids into the phloem of a new host plant (Agrios, 1997). Usually these insect vectors do not transmit phytoplasmas transovarially, although two exceptions have been reported: aster yellows and mulberry dwarf phytoplasmas (Alma *et al.*, 1997; Kawakita *et al.*, 2000).

Important diseases caused by Phytoplasmas are:

Disease Name	Species
Aster yellows	<i>Ca. Phytoplasma asteris</i> <i>Ca. Phytoplasma japonicum</i>
Peanut witch's broom	<i>Ca. Phytoplasma aurantifolia</i>
X-disease	<i>Ca. Phytoplasma pruni</i>
Coconut lethal yellowing	<i>Ca. Phytoplasma palmarum</i> <i>Ca. Phytoplasma castaneae</i> <i>Ca. Phytoplasma cocosnigeriae</i>
Elm yellows	<i>Ca. Phytoplasma ziziphi</i> <i>Ca. Phytoplasma vitis</i> <i>Ca. Phytoplasma ulmi</i>
Clover proliferation	<i>Ca. Phytoplasma trifolii</i>
Ash yellows	<i>Ca. Phytoplasma fraxini</i>
Luffa witch's-broom	<i>Ca. Phytoplasma luffae</i>
Pidgeon pea witch's broom	<i>Ca. Phytoplasma phoenicium</i>
Apple proliferation	<i>Ca. Phytoplasma mali</i> <i>Ca. Phytoplasma pyri</i> <i>Ca. Phytoplasma prunorum</i> <i>Ca. Phytoplasma spartii</i> <i>Ca. Phytoplasma rhamni</i> <i>Ca. Phytoplasma allocasuarinae</i>
Rice Yellow Dwarf	<i>Ca. Phytoplasma oryzae</i>

Control of disease

There is no general and efficient methods to control mollicutes. Today, control of plant pathogenic mollicutes is based, when possible, on (i) planting mollicute-free stocks, (ii) removal of sources of infections (iii) control of vectors (iv) and use of tolerant (less susceptible) varieties. Planting mollicute-free stock is the easiest step to achieve. Mollicutes are not seed-transmitted and can be eliminated from contaminated propagating material by meristem cultures or thermotherapy.

Fungi – A general account and reproduction

What are fungi? Their principle types

The term **fungi** is defined in two ways in modern botanical literature.

1. **In non-taxonomic way:** According to this explanation, the fungi are heterotrophic eukaryotes, showing sporulative multiplication, filamentous growth and absorptive mode of nutrient acquisition (osmotrophy).
2. **In a taxonomic sense:** This explanation attempts at creating a phylogenetically cohesive group. According to this, Fungi are heterotrophic eukaryotes having originated from the protistan group Choanoflagellata, showing chitinous cell wall, filamentous growth (if not unicellular), sporulative multiplication and absorptive mode of nutrient acquisition (osmotrophy).

The above taxonomic definition of fungi applies only to the members of the Kingdom Mycota within the domain Eukarya. It is noteworthy that the Kingdom Mycota, as it is currently organized, does not include the Oomycetes and the Myxomycetes. In this kingdom, there are seven phyla considered currently (Hibbett, D.S., et al. (2007). "A higher level phylogenetic classification of the Fungi" in *Mycological Research* Volume 111, Issue 5, May 2007).

1. Phylum Chytridiomycota
2. Phylum Blastocladiomycota
3. Phylum Neocallimastigomycota
4. Phylum Zygomycota
5. Phylum Glomeromycota
6. Phylum Ascomycota
7. Phylum Basidiomycota

Because of some similarities in morphology and lifecycle, Myxomycetes and Oomycetes were formerly classified in the kingdom Fungi. Molecular evidences reveal that neither water molds (Oomycetes) nor slime molds (Myxomycetes) are closely related to the true fungi, and, therefore, taxonomists no longer group them in the kingdom Fungi. Now slime molds are grouped in the Amoebozoa and water molds are grouped in the Stramenopila kingdom. These organisms are now treated as Fungi-like organisms, rather than true Fungi.

The salient features of the fungi

The salient features of the fungi include the following:

1. Occurring worldwide especially under somewhat humid conditions and living for the most part in soil, dead matter, and as symbionts of plants, animals, or other fungi.
2. Largely invisible to the unaided eye due to very small size
3. Eukaryotic organization of cells
4. A chitinous cell wall
5. Majority of species grow as multicellular / multinucleate filaments called hyphae forming a mycelium; some fungal species also grow as single cells.
6. Heterotrophic behaviour with absorptive mode of food acquisition.
7. All the phyla show monoploid genetic constitution in the somatic stage.
8. Asexual reproduction of the fungi is commonly via spores
9. Sexual reproduction is mediated by gametic fusion, gametangial fusion, spermatization or even somatogamy. Sexual reproduction with meiosis exists in all fungal phyla, except the Deuteromycota.
10. Many fungal species have elaborate vegetative incompatibility systems that allow mating only between individuals of opposite mating type, while others can mate and sexually reproduce with any other individual or itself. Species of the former mating system are called heterothallic, and of the latter homothallic.
11. Some species have lost the ability to form reproductive structures, and propagate solely by vegetative growth.

12. Fungi produce several secondary metabolites functioning as defensive compounds or for niche adaptation. Many such products are of commercial interest.

A short account of different Fungal phyla

1. The Chytridiomycota are commonly known as chytrids. Chytrids produce zoospores that are capable of active movement through aqueous phases with a single flagellum.
2. The Blastocladiomycota were previously considered a taxonomic clade within the Chytridiomycota. Recent molecular data and ultrastructural characteristics, however, place the Blastocladiomycota as a sister clade to the Zygomycota, Glomeromycota, and Dikarya (Ascomycota and Basidiomycota). The blastocladiomycetes are fungi that are saprotrophs and parasites of all eukaryotic groups and undergo sporic meiosis unlike their close relatives, the chytrids, which mostly exhibit zygotic meiosis.
3. The Neocallimastigomycota were earlier placed in the phylum Chytridiomycota. Members of this small phylum are anaerobic organisms, living in the digestive system of larger herbivorous mammals and possibly in other terrestrial and aquatic environments. They lack mitochondria but contain hydrogenosomes of mitochondrial origin. As the related chytrids, neocallimastigomycetes form zoospores that are posteriorly uniflagellate or polyflagellate.
4. The Zygomycota contain the taxa, Zygomycetes and Trichomycetes, and reproduce sexually with meiospores called zygospores and asexually with sporangiospores. Black bread mold (*Rhizopus stolonifer*) is a common species that belongs to this group; another is *Pilobolus*, which is capable of ejecting spores several meters through the air. Medically relevant genera include *Mucor*, *Rhizomucor*, and *Rhizopus*. Molecular phylogenetic investigation has shown the Zygomycota to be a polyphyletic phylum with evidence of paraphyly within this taxonomic group.
5. Members of the Glomeromycota were once considered within Zygomycota. They are fungi forming arbuscular mycorrhizae with higher plants. Only one species has been observed forming zygospores; all other species solely reproduce asexually. The symbiotic association between the Glomeromycota and plants is ancient, with evidence dating to 400 million years ago.
6. The Ascomycota, commonly known as sac fungi or ascomycetes, constitute the largest taxonomic group within the Eumycota. These fungi form meiotic spores called ascospores, which are enclosed in a special sac-like structure called an ascus. This division includes morels, a few mushrooms and truffles, single-celled yeasts (e.g., of the genera *Saccharomyces*, *Kluyveromyces*, *Pichia*, and *Candida*), and many filamentous fungi living as saprotrophs, parasites, and mutualistic symbionts. Prominent and important genera of filamentous ascomycetes include *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps*. Many ascomycetes species have only been observed undergoing asexual reproduction (called anamorphic species), but molecular data has often been able to identify their closest teleomorphs in the Ascomycota. Because the products of meiosis are retained within the sac-like ascus, several ascomycetes have been used for elucidating principles of genetics and heredity (e.g. *Neurospora crassa*).
7. Members of the Basidiomycota, commonly known as the club fungi or basidiomycetes, produce meiospores called basidiospores on club-like stalks called basidia. Most common mushrooms belong to this group, as well as rust (fungus) and smut fungi, which are major pathogens of grains. Other important Basidiomycetes include the maize pathogen, *Ustilago maydis*, human commensal species of the genus *Malassezia*, and the opportunistic human pathogen, *Cryptococcus neoformans*.

Fungal Reproduction

Each of the four fungal groups is characterized by differences in their life cycles. All fungi are characterized by having a period of vegetative growth where their biomass increases. The length of time and the amount of biomass needed before sporulation can vary significantly from one species to another.

Almost all fungi reproduce by the production of spores, but a few have lost all sporing structures and are referred to as *mycelia sterilia*. Different types of spore are produced in different parts of the life cycle.

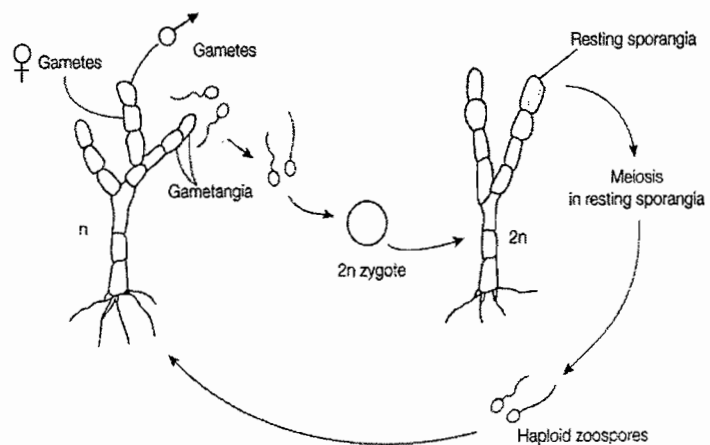


Figure 2: Reproduction in Chytridiomycota

Reproduction in Chytridiomycota

Chytrids are lower fungi, quite distinct from other fungi as they have extremely simple thalli and motile zoospores. Some species within this group can be so simple that they consist of a single vegetative cell within (endobiotic) or upon (epibiotic) a host cell, the whole of which is converted into a sporangium, a structure containing spores. These types are termed holocarpic forms.

Other members of this group have a more complex morphology, and have rhizoids and a simple mycelium.

Asexual reproduction

Asexual reproduction in the chytrids is by the production of motile zoospores in sporangia that are delimited from the vegetative mycelium by complete septae. The zoospores have a single, posterior flagellum.

Sexual reproduction

Sexual reproduction occurs in the chytrids by either somatic fusion of haploid cells, or two different mating-type mycelia, or fusion of two motile gametes, or fusion of one motile gamete with a non-motile egg (Fig. 1). The production of diploid spores from the zygotic cell occurs after the sexual fusion. The resulting spore may undergo meiosis to produce a haploid mycelium or it may germinate to produce a diploid vegetative mycelium, which can undergo asexual reproduction by production of diploid zoospores. The diploid mycelium can also produce resting sporangia in which meiosis occurs, generating haploid zoospores that germinate to produce haploid vegetative mycelium.

Reproduction in Zygomycota

Asexual Reproduction

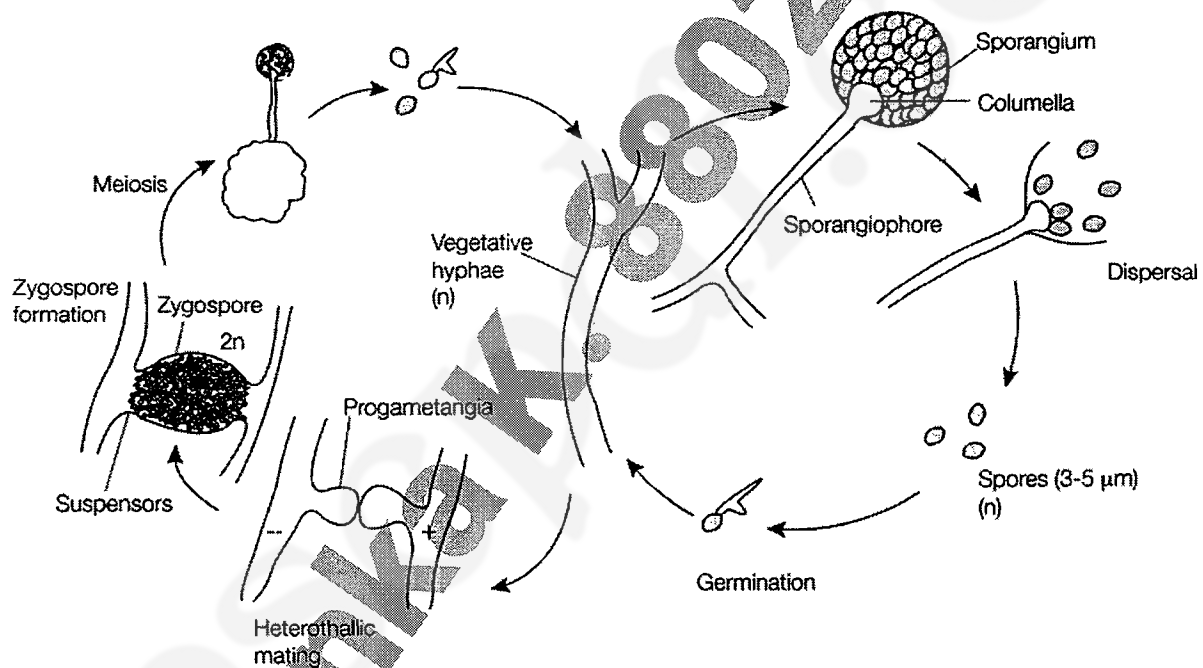


Figure 2: Reproduction in a Member of Zygomycota

In this phylum of lower fungi, asexual reproduction begins with the production of aerial hyphae. The tip of an aerial hypha, now called a sporangiophore, is separated from the vegetative hyphae by a complete septum called a columella. The cytoplasmic contents of the tip differentiate into a sporangium containing many asexual spores. The spores contain haploid nuclei derived from repeated mitotic divisions of a nucleus from the vegetative mycelium. Dispersal of the spores is by wind or water (Fig. 2).

Sexual Reproduction

In sexual reproduction, two nuclei of different mating types fuse together within a specialized cell called a zygospore (Fig. 2). In some species the different mating-type nuclei may be within one mycelium (*homothalaxis*). In other species, two mycelia with different mating-type nuclei must fuse (*heterothalaxis*). In both cases, fusion occurs between modified hyphal tips called *progametangia*, which once fused are termed the *zygospore*.

Within the developing zygospore meiosis occurs; usually three of the nuclear products degenerate, leaving only one nuclear type present in the germinating mycelium.

Reproduction in Ascomycota

Asexual Reproduction

The vegetative stage of the Ascomycete life cycle is accompanied or followed by asexual sporulation by the production of single spores called conidia from the tips of aerial hyphae called *conidiophores* (Fig. 3). There are two possibilities in the mode of conidiospore formation.

1. The spores can be delimited by a complete transverse wall formation followed by spore differentiation (Fig. 3a) termed *thallic spore formation*.
2. More usually, the spores are formed by the extrusion of the wall from the hyphal tip, termed *blastic spore formation* (Fig. 3b).

These conidiospores are mostly single celled and contain one haploid nucleus, but in some cases they can be multinucleate and contain several haploid nuclei produced by mitosis.

Spores can be produced from single, unprotected conidiophores or they can be produced from aggregations that are large enough to be seen with the naked eye (Fig. 3c).

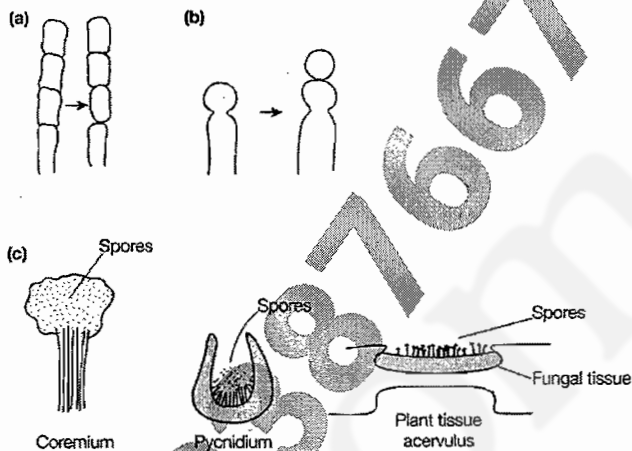


Figure 3: Asexual sporulation in Ascomycota

There are several modes of conidiophores aggregations.

- The conidiophores can aggregate into stalked structures where the spores produced are exposed at the top (*synnema* or *coremia*).
- In some cases, varying amounts of sterile fungal tissue can protect the conidia, as in the flaskshaped *pycnidia*.
- Some species produce conidia in plant tissue, and the conidial *acervulus*.

Sexual Reproduction

Sexual reproduction in Ascomycota occurs after fusion of different mating-type mycelia by any one of the following three modes.

- Mating cell fusion (in Yeasts)
- Gametangial contact
- Spermatisation
- Somatogamy

After sexual fusion generally a *dikaryotic phase* exists for a considerable period of time in most of the advanced member of this phylum. The fusion between the two haploid nuclei forms the diploid zygotic nucleus.

A transient diploid phase is rapidly followed by the formation of ascospores within sac-shaped asci differentiated from modified hyphal tips. In the initial stages of ascus development hooked hyphal tips form, called *croziers* or *shepherds' crooks* because of their shape. They have distinctive septae at their base which insures that two different mating-type nuclei are maintained in the terminal cell. Formation of the septae is coordinated with nuclear division (Fig. 4). In yeasts all these events occur within one cell, after fusion of two mating-type cells, the whole cell being converted into an ascus.

In more complex Ascomycetes many asci form together, creating a fertile tissue called a *hymenium*. In some groups the hymenium can be supported or even enclosed by large amounts of vegetative mycelium. The whole structure is called a fruit body or *sporocarp* and is used as a major taxonomic feature (Fig. 5). They can become large enough to

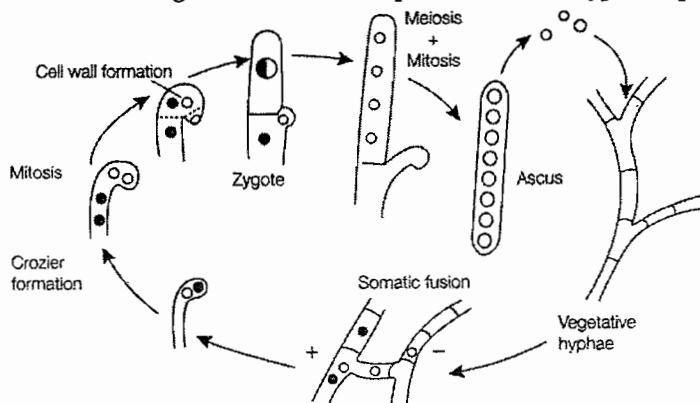


Figure 4: Sexual process in Ascomycetous Fungi

be seen with the naked eye. Flaskshaped sexual reproductive bodies are called perithecia, cup-shaped bodies are called apothecia and closed bodies are called cleistothecia. These structures have evolved to protect the asci and assist in spore dispersal, but the hymenium itself is unaffected by the presence of water.

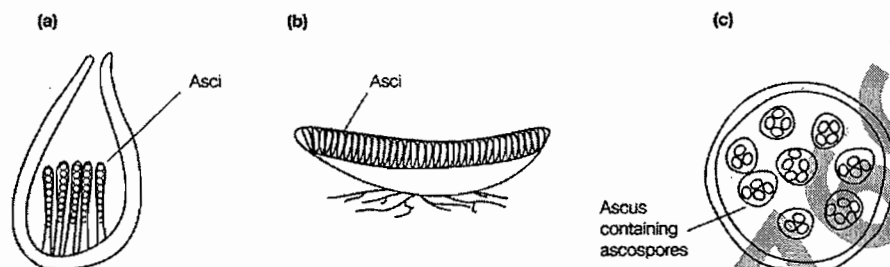


Figure 5: Three major types of sporocarps in Ascomycota. (a) Perithecium (b) Apothecium (c) Cleistothecium

Reproduction in Basidiomycota

This group of fungi are characterized by the most complex and large structures found in the fungi. They are also distinctive in that they very rarely produce asexual spores.

Much of the life cycle is spent as vegetative mycelium, exploiting complex substrates. A preliminary requisite for the onset of sexual reproduction is the acquisition of two mating types of nuclei by the fusion of compatible hyphae. The nuclei from two mating-type are held within every hypha compartment for extended periods of time. This is termed a *dikaryotic state*, and its maintenance requires elaborate septum formation during growth and nuclear division.

Onset of sexual-spore formation is triggered by environmental conditions and begins with the formation of a fruit body primordium. Dikaryotic mycelium expands and differentiates to form the large fruit bodies or *sporocarps*, which we recognize as mushrooms and toadstools. Diploid formation and meiosis occur within a modified hyphal tip called a *basidium* (Fig. 6).

Four spores are budded from the basidium. Basidia form together to create a hymenium which is highly sensitive to the presence of free water. The hymenium is distributed over sterile, dikaryotic supporting tissues which protect it from rain.

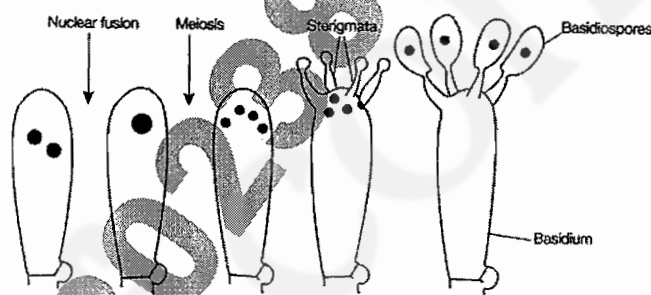


Figure 6: Basidium Formation

Archaea

Introduction

The Archaea are unique micro-organisms. While prokaryotic in the cytological sense (that is they lack a true, membrane delimited nucleus), they are actually more closely related to eukaryotes than to the bacteria. Evolutionarily, the Archaea represent the oldest surviving group of organisms on the earth. Many Archaea are known to be extremophiles.

They were properly characterized by **Carl Woese** and **George Fox** in 1977.

Because of prokaryotic cellular organization, earlier they were considered bacteria. However, using 16-S ribosomal RNA sequence as an evolutionary measure, Woese *et al.* established the uniqueness of Archaea vis-à-vis the bacteria.

After that, these organisms are grouped as a distinct domain called Archaea.

Systematic position

Under 5 Kingdom Systems

Robert Whittaker in 1969 proposed a **5 Kingdom Systematic plan** of life forms. The members of Archaea were placed in the **Kingdom Monera** that included all the prokaryotes.

In Kingdom Monera, Whittaker recognized 3 principal groups:

1. Archaeobacteria
2. Eubacteria
3. Cyanobacteria

Thus the organisms which constitute the Archaea were placed in the sub-kingdom Archaeobacteria of Monera by Whittaker. Many biologists did not accept Whittaker's system, primarily because it did not distinguish bacteria from Archaea.

Under 3 Domain System

The three-domain system is a biological classification introduced by **Carl Woese** in 1990. Woese and collaborators used comparative rRNA studies to group all living organism into three domains

- **Bacteria**-comprise the vast majority of procaryotes; cell walls contain muramic acid; membrane lipids contain ester-linked straight-chain fatty acids
- **Archaea**-procaryotes that lack muramic acid, have lipids with ether-linked branched aliphatic chains, lack thymidine in the T arm of tRNA molecules, have distinctive RNA polymerases, have introns in rRNA and tRNA genes, and have ribosomes with a different composition and shape than those observed in Bacteria
- **Eucarya**-have a more complex membrane-delimited organelle structure

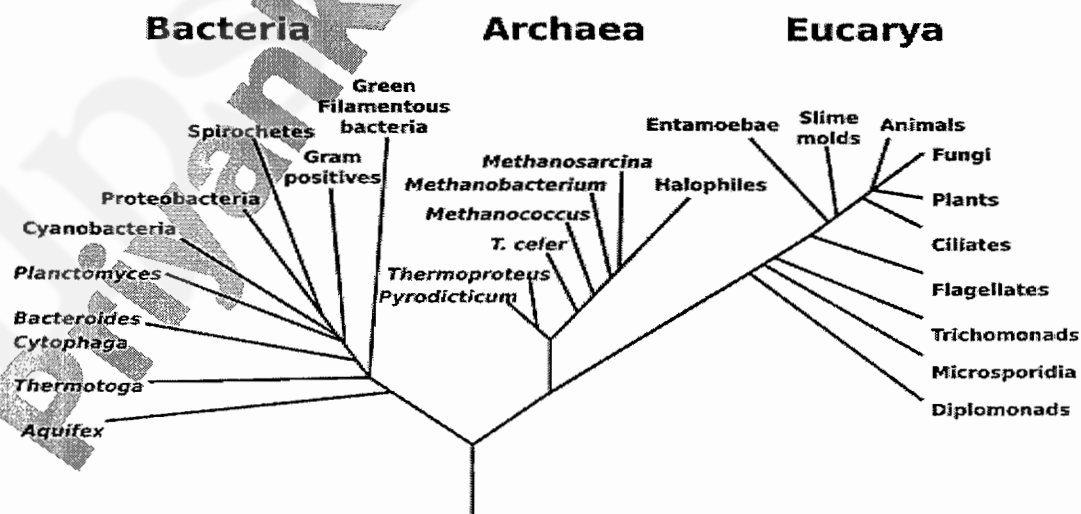


Figure 1: 3 Domain System of Carl Woese

Why are the Archaea not placed with the Bacteria?

Archaea are similar to the bacteria in many aspects of cell structure and metabolism. However, in a number of molecular structures and processes, the Archaea do not show the typical bacterial features, but are extremely similar to those of eukaryotes or unique in their own right. For instance:

1. They are often found in extreme aquatic and terrestrial habitats which can not be tolerated by bacteria or the eukaryotes.
2. Archaeal cell wall chemistry is different from that of bacteria
 - a. Lacks muramic acid and D-amino acids and therefore is resistant to lysozyme and β -lactam antibiotics
 - b. Some have pseudomurein
 - c. Others have different polysaccharides
3. Archaeal membrane lipids have branched hydrocarbons attached to glycerol by ether links rather than straight-chain fatty acids attached to glycerol by ester links as seen in Bacteria and Eucarya.
4. Often, more complex tetraether structures are also found. Membranes of extreme thermophiles are almost completely tetraether monolayers.
5. Many Archaeal lipids are based upon the isoprenoid sidechain. This is a five-carbon unit that is also common in rubber and as a component of some vitamins common in bacteria and eukaryotes. However, only the archaea incorporate these compounds into their cellular lipids.
6. The archaeal chromosomes are considerably smaller than the bacteria.
7. The Archaeal chromosomes contain histone like proteins.
8. Genomic analysis suggests that the Archaea are distinctive genotypically also.
9. Archaeal mRNA is like that of bacteria but rRNA and tRNA often contain introns, which are spliced out.
10. Ribosomes are 70S type but with different morphological and physiological properties than bacterial and eucaryotic ribosomes.
11. Archaeal RNA polymerase enzymes are more similar to eukaryotic enzymes than to bacterial enzymes.
12. The transcription machinery also shows TATA Binding Protein and Transcription Factor IIB (otherwise seen only in eukaryotes)
13. Translation Factor Proteins are more similar to eukaryotes.
14. There is a use of Methionine in the initiator tRNA and not Formylated Methionine, as found in the eubacteria
15. The translation machinery is poisoned by Diphtheria toxin, which never affects the bacterial cells.
16. Archaea do not use the Embden-Meyerhof pathway for glucose catabolism; however they frequently use a reversal of that pathway for gluconeogenesis as seen in higher eukaryotes.
17. Archaeans also have flagella that are notably different in composition and development from the superficially similar flagella of bacteria.

Comparison of the 3 domains

Characteristic	Eukarya	Bacteria	Archaea
Habitat	Mesophilic with exceptions	Mesophilic with exceptions	Extremophilic with exceptions
16S rRNA sequence	Unique	Unique	Unique
Cell wall component	Polysaccharides in plants; none in animals; chitin in fungi	Peptidoglycan or murein	Pseudopeptidoglycan or pseudomurein
Cell membrane	Straight chain fatty acids linked to glycerol by ester linkages	Straight chain fatty acids linked to glycerol by ester linkages	Branched chain hydrocarbons linked to glycerol by ether linkages
Sensitivity to diphtheria toxin	Sensitive	Not sensitive	Sensitive
Sensitivity to chloramphenicol	Not sensitive	Sensitive	Not sensitive
Halorhodopsin (light sensitive pigment)	Rhodopsin, a similar pigment, present	Absent	Halorhodopsin in halophilic Archaea
Unusual Coenzymes	Absent	Absent	Present
Genome organization	Eukaryotic, with nucleus, and chromosomes etc.	DNA circular, naked.	DNA circular, naked
DNA binding proteins like histones	Histones present	Histones absent	Histone-like proteins present in some Archaea
DNA polymerases	Eukaryal	Bacterial	Polymerases with primary protein sequences resembling Eukarya
RNA polymerases	Eukaryal	Bacterial	More complex than bacterial, more close to Eukaryal
Promoters	Eukaryal	Bacterial	More like Eukaryal
Polypeptide chain initiation	Methionine	N-formyl Methionine	Methionine
Coenzyme M	Absent	Absent	Present
Introns	Present	Absent	Present in rRNA and tRNA genes
Translation signals	Signals unique to Eukarya	Signals unique to bacteria	Signals resemble those in bacteria

Origin of Archaea

We can conclude from this curious mix of characteristics that:

1. Many traits found in the bacteria first appeared in the ancestors of all the present-day groups.
2. The split leading to the archaea and the eukaryotes occurred after the bacteria had already emerged.
3. However, the acquisition by eukaryotes of mitochondria (probably from an ancestor of today's rickettsias) and chloroplasts (from cyanobacteria) occurred after their line had diverged from the archaea.
4. The archaean ability to live in extreme environments and autotrophism; that is, their ability to make food using materials (H_2 , S, CO_2) in the earth's crust have suggested that the archaea may be the little-changed descendants of the first forms of life on earth.

Groups of Archaea

The new Second edition of **Bergey's Manual** divides the archaea into two phyla: Euryarchaeota and Crenarchaeota. However, modern microbiologists identify **four phyla of Archaea** [Brock's Microbiology (2006, 11th Edition)].

The four archaeal phyla are:

1. **Euryarchaeota**
2. **Crenarchaeota**
3. **Korarchaeota**
4. **Nanoarchaeota**

The **Euryarchaeota** are a major group of Archaea. They are separated from the other archaeans based mainly on rRNA sequences.

Extremophilic behaviour of Archaea

The Archaea have also been called **Extremophiles** in recognition of the extreme environments in which they have been found. They can be:

- **thermophiles**, which live at high temperatures;
- **hyperthermophiles**, which live at extremely high temperatures (presently known up to 121°C);
- **thermoacidophiles**, which prefer hot and acidic habitats.
- **psychrophiles**, which prefer very cold habitats such as in the Antarctic grows between 0°– 4°C);
- **halophiles**, which live in very saline environments (like the Dead Sea);
- **acidophiles**, which live at low pH (as low as pH 1 and they can die at pH 7);
- **alkaliphiles**, which thrive at a high pH.
- **barophiles**, which live in environments characterized by high gas or liquid pressure; synonymous with piezophile.

Some well known examples of extreme loving behaviour are:

- *Archaeoglobus fulgidus* is found in oil wells.
- *Halobacteria* are extreme salt-loving microbes that give a pink tinge to salt water evaporation ponds, the Dead Sea and salted fish.
- *Pyrolobus fumarii* led scientists to extend the upper temperature limit for life to 113 degrees Celsius (235.4 degrees Fahrenheit).
- *Pyrococcus furiosus* can survive at 105 degrees Celsius
- *Sulfolobus acidocaldarius* prefers sulfur rich acidic medium.
- The two *Picrophilus* species are the most highly acidophilic organisms known to science. They have an optimal pH requirement of 0.7, can still grow at a pH of -0.06 and die at pH values of less than 4.0.
- *Ferroplasma* is an iron oxidising-nitrate reducing organism of shallow marine hydrothermal vents (Fe^{2+} to Fe^{3+} , NO_3^- to NO_2^- and/or NO).

The cellular basis of extreme tolerance

Extremophilic microorganisms have adapted their molecular machinery to grow and thrive under the most adverse environmental conditions. Some well characterized mechanisms are as follows:

- Membrane Lipids of Archaea have branched hydrocarbons attached to glycerol by ether links. In other organisms, it is usually straight-chain fatty acids attached to glycerol by ester links. Ether links provide the Archaeal membranes much greater stability even under high temperatures.
- Archaeal Membranes contain polar lipids such as phospholipids, sulfolipids, and glycolipids and also contain nonpolar lipids (7-30%), which are usually derivatives of squalene. It is a triterpene hydrocarbon and biochemical precursor to the whole family of steroids. The derivatives of squalene keep the membrane stable under varying temperature conditions.
- Membranes of extreme thermophiles are almost completely tetraether monolayers that confers the greatest degree of stability.
- Histone like proteins stabilize their DNA under a variety of conditions
- The extremophilic proteins adopt various strategies and adaptation to extreme temperature and solvent conditions, through new combinations of same weak electrostatic and hydrophobic interactions among the ordinary amino acid residues which are found in mesophilic proteins.

- The archaea found in extremely saline environments such as the Great Salt Lake in the U.S. and the Dead Sea, maintain osmotic balance with their surroundings by building up the solute concentration within their cells.

Applications

1. *Sulfolobus acidocaldarius* is used to leach copper and iron from ore.
2. Because the extremophiles have enzymes that can function at high temperatures, considerable effort is being made to exploit the archaea for commercial processes such as providing:
 - a. Enzymes to be added to detergents (maintain their activity at high temperatures and pH)
 - b. Enzymes potentially useful in the PCR:
 - Pfu: From *Pyrococcus furiosus*. Appears to have the lowest error rate of known thermophilic DNA polymerases.
 - Vent: From *Thermococcus litoralis*; also known as Tli polymerase. Half-life at 95 C is approximately 7 hours.
 - c. Enzymes used in dairy industry to digest fats in churning vessels
 - d. An enzyme to convert corn starch into dextrins.
3. Archaea may also be enlisted to aid in cleaning up contaminated sites, e.g., petroleum spills.

Applications of microbes in agriculture, industry and medicine

Microbes in agriculture

Microbes include bacteria, viruses, protists, microalgae and fungi. Laypersons often think of microbes as harmful agents destructive to crops or livestock, but many microbes are beneficial. Besides being important in biogeochemical cycling of nutrients, microbes play vital role in maintenance of soil fertility and in crop protection. In fact, it would be appropriate to say that the microorganisms have been friends of farmers even before the humanity could realize it.

Some beneficial aspects are enumerated below.

1. Soil microbes (bacteria and fungi) are essential for decomposing organic matter and recycling old plant material. Decomposition of organic matter involves vital conversions like the one from amino acids to ammonia and the one from ammonia to nitrate – which provide the crucial link to nitrogen availability to the land plants.
2. Soil Fertility - Nitrogen-fixing microbes are being exploited as biofertilisers. Potential of nitrogen-fixing organisms like *Rhizobium*, *Azotobacter*, *Beijerinckia*, *Azospirillum*, *Cyanobacteria*, such as species of *Aulosira*, *Anabaena*, *Nostoc*, *Plectonema*, *Scytonema*, *Tolypothrix*, and *Azalia* as biofertilisers has been exploited so as these could serve an alternative to chemical fertilisers. Many brands of Rhizobial inoculants are already in market today in the country. Several organisation and manufacturers are producing huge quantities of *Rhizobium* culture in the country.
3. Through recombinant DNA technology efforts have been made to introduce nitrogen-fixing genes (*nif* genes) into wheat, corn, rice, etc. Plasmids of the bacterium, *E. coli* and yeast are being worked out for such a possibility. Hybrid *E. coli* plasmid cloned with *nif* genes of a nitrogen-fixing bacterium, *Klebsiella pneumoniae* and hybrid yeast plasmids are then integrated.
4. Mycorrhizae, both ecto and endomycorrhiza help in uptake of N, P, K and Ca. They particularly help in phosphorous nutrition. Mycorrhizae also offer better water uptake and protection from several pathogenic agents such as harmful fungi and nematodes.
5. Some soil bacteria also form relationships with plant roots that provide important nutrients like nitrogen or phosphorus. The ability of a few soil microorganisms to convert insoluble forms of phosphorus to an accessible form is an important trait in plant growth-promoting bacteria for increasing plant yields. The use of phosphate solubilizing bacteria as inoculants increases the P uptake by plants. Four strains namely, *Arthrobacter ureafaciens*, *Phyllobacterium myrsinacearum*, *Rhodococcus erythropolis* and *Delftia* sp. have been widely reported as phosphate solubilizing bacteria (PSB).
6. Several microbes (viruses bacteria, and fungi) are being developed as suitable biopesticides for management of insect and nematodal pests. Some fungi have good potential of their use as bionematicides to control nematodal pests of vegetables, fruit and cereal crops. Some bacterial and fungal products are also in use to control diseases of roots and shoots of plants.

Some Commercial Microbial Pesticides include the following.

Microbe used as pesticide	Registered product, if any	Target Disease/pest being controlled
Viruses		
NPV	ELCAR	Tobacco budworm
NPV	GYPCHK	Gypsy moth
CPV	MATSUKEMIN	Pine caterpillar
NPV	VIRTUSS	Douglas fir
Bacteria		
<i>Bacillus thuringiensis</i>	THURICIDE	A range of insects
<i>Agrobacterium radiobacter</i> K84	GALLTROL-A	Crown gall of stone fruits.

<i>Pseudomonas</i> sp. and <i>Enterobacter</i> sp.	DAGGER-G	Damping off of above-ground parts of cotton seedlings
<i>Pseudomonas</i> sp. and <i>Bacillus</i> spp.	QUANTUM-4000	Wilt of cereals and vegetables
Fungi		
<i>Peniophora gigantea</i>		Root-rot of pine stumps
<i>Beauveria bassiana</i>	BOVERIN	Range of insects
<i>Metarhizium anisopliae</i>	METABIOL, METAQUINO	Range of insects
<i>Aschersonia aleyrodis</i>	ASERONIJA	Glasshouse white fly

- Several fungi have been found very useful in the control of troublesome weeds of crop fields. Registered products are available in market for use in several countries.
- Fungi can colonize parts of plants and provide many benefits, including drought tolerance, heat tolerance, resistance to insects and resistance to plant diseases. In a recent study in Yellowstone National Park, USA, some plants were found to grow very well in soil temperatures of 115°F. These plants were found to be colonized by a fungus. Without the fungus, the plant could not tolerate the heat. It was further found that there was a virus in the fungus. Without the virus, the fungus could still colonize the plants, but it no longer conferred tolerance to heat. When the scientists reintroduced the virus, it restored heat tolerance.

Microbes in medicine

Microbial technology has contributed to the production of various chemicals such as antibiotics, vitamins, steroids and vaccines, thus being the mainstay of pharmaceutical industry. Antibiotics production itself is a major industry. Some of the important microbial processes of the production of pharmaceuticals are discussed here.

Antibiotics

Antibiotics are antimicrobial agents of microbial origin. Most antibiotics are industrially produced by microbial fermentation though some are now synthetically produced (e.g., chloramphenicol). Some important antibiotics and the microorganisms producing them are listed in table below.

Vitamins

One of the most essential vitamins produced through microbial fermentation is Vitamin B₁₂ (Cyanocobalamin). Vitamin B₁₂ can be produced as a by-product of Streptomycin and Aureomycin fermentations by *Streptomyces* species. A cobalt salt is added to the fermentation medium as a precursor to increase the yield of Vitamin B₁₂. However, the accumulation of the vitamin does not adversely affect the growth of *Streptomyces*. Some important vitamins and the microorganisms producing them are listed in table below.

Antibiotic	Producing microorganism
Penicillin	<i>Penicillium chrysogenum</i>
Cephalosporin	<i>Cephalosporium acremonium</i>
Griseofulvin	<i>Penicillium griseofulvum</i>
Chloramphenicol	<i>Streptomyces venezuelae</i>
Tetracyclines	
Tetracycline	<i>Streptomyces aureofaciens</i>
Chlortetracycline	<i>S. aureofaciens</i>
Oxytetracycline	<i>S. rimosus</i>
Polypeptides	
Polymyxin-B	<i>Bacillus polymyxa</i>
Bacitracin	<i>B. licheniformis</i>
Glutarimides	
Cycloheximide	<i>Streptomyces griseus</i>
Aminoglycosides	
Streptomycin	<i>Streptomyces griseus</i>
Kanamycin	<i>S. kanamyceticus</i>
Neomycin	<i>S. fraadiae</i>
Polynes	
Nystatin	<i>Streptomyces noursei</i>
Hamycin	<i>Streptomyces</i> sp.
Aureofungin	<i>Streptomyces</i> sp.
Amphoterecin-B	<i>S. nodosus</i>
Macrolides	
Erythromycin	<i>Streptomyces erythreus</i>
Oleandomycin	<i>S. antibioticus</i>
Carbomycin	<i>S. halstedii</i>
Novobiocin	<i>Streptomyces niveus</i>
Blasticidin-S	<i>Streptomyces</i> sp.
Vira-A	<i>Streptomyces antibioticus</i>
(Adenine arabinoside)	

Vitamin	Organism	Medium	Fermentation conditions	Yield
Riboflavin	<i>Ashbya gossypii</i>	Glucose, collagen, Soya oil, Glycine	6 days at 36°C Aerobic	4.25 g/L
L-Sorbose (for Vitamin C)	<i>Gluconobacter oxidans</i> sub sp. <i>suboxidans</i>	30% Cornsteep, D. Sorbitol.	45 hrs. at 30°C Aerobic	70% (based on substrate used)
5-Ketogluconic acid (for Vitamin C)	<i>Gluconobacter oxidans</i> sub sp. <i>suboxidans</i>	Glucose, CaCO ₃ cornsteep.	33 hrs. at 30°C; Aerobic	100% (based on substrate used)
Vitamin B ₁₂	<i>Propionibacterium shermanii</i>	Glucose, Cornsteep, ammonia, cobalt, pH 7.0	3 days at 30°C anaerobic and 4 days aerobic	23 mg/L

Steroids

Steroid hormones have found a number of therapeutic applications in recent years. Cortisone as been found to relieve pain due to rheumatoid arthritis. Various other derivatives of cortisone have been useful in alleviating allergic and inflammatory responses of the human body. Another area where steroid hormones have found use is in controlling fertility. Some of these steroids are therefore used in birth control pills. The importance of steroid hormones in the pharmaceutical industry is, therefore, immense.

Microbial biotransformation of steroids is very important in the pharmaceutical industry. For example, cortisone can be synthesised chemically from deoxycholic acid but the process requires 37 steps, many of which must be carried out under extreme conditions of temperature and pressure with the resulting product costing over \$200 per gram. The most difficult is introduction of oxygen atom at number 11 position of the steroid ring, but this can be accomplished by some microorganisms. The fungus, *Rhizopus nigricans* for example hydroxylates progesterone, forming another steroid with the introduction of oxygen at the number 11 position.

The fungus *Cunninghamella blakesleeana* similarly can hydroxylate the steroid cortexolone to form hydrocortisone with the introduction of oxygen at number 11 position. Other transformations of the steroid nucleus carried out by microbes include hydrogenations, dehydrogenations, epoxidations and removal and addition of the side chains.

In a typical steroid transformation process, the microbe, such as *Rhizopus nigricans* is grown in a fermentation tank using an appropriate growth medium and incubation conditions to achieve a high biomass. In most cases agitation and aeration are done to have rapid growth.

After the growth of the microbe, the steroid to be transformed is added (as progesterone here) to the fermentor containing *R.nigricans* that has been growing for one day or so and the steroid is hydroxylated at number 11 to form 11- α - hydroxyprogesterone.

Product is recovered by extraction with methylene chloride or other solvents, purified chromatographically and recovered by crystallization.

Human Protein Synthesis- Genetic engineering has expanded the industrial applications of microorganisms including production of human proteins. By using recombinant DNA technology, human DNA sequences that code for various proteins have been incorporated into the genomes of bacteria. By growing these recombinant bacteria in fermentors, human proteins could be produced commercially.

Human insulin, for instance, is produced by a recombinant *E. coli* strain and marketed as humulin. Other strains are used to produce human growth hormone, tumor necrosis factor (TNF), interferon (human recombinant beta interferon-trade name, Betaseron), and inter- leukin-2 (human recombinant interleukin-2, trade name- Prolcukin).

Humulin is used in treatment of diabetes in individuals allergic to insulin harvested from cattle. Human growth factor is used in treatment of dwarfism, and interleukin- 2, interferon and TNF are important components of human immune system.

Vaccines Preparations - Production of vaccines involves growing the microbes possessing the antigenic properties needed to elicit a primary immune response. Mutant strains and attenuated or inactivated virulent pathogens (without removing antigens) are used for producing vaccines. Prophylactic treatment of serious pathogenic viruses and bacteria could become possible only by vaccines.

Viruses are grown in embryonated eggs or tissue cultures. The rabies vaccine, produced earlier in embryonated duck eggs with painful side effects has now been replaced by a vaccine produced in human fibroblast tissue cultures. More information on vaccines including genetically engineered vaccines on "General (nonspecific) and Immune (specific) Response".

Microbes in industry

Industrial microbiology and biotechnology involve the use of microorganisms to achieve specific goals. Biotechnology has developed rapidly due to the genetic modification of microorganism, particularly by recombinant DNA technology.

The following are the important aspects of Industrial Microbiology and Biotechnology.

1. Choosing Microorganisms for Industrial Microbiology and Biotechnology
 - a. Major sources of microorganisms for use in industrial processes are soil, water, and spoiled bread and fruits; and only a minor portion of microbial species in most environments have been identified; therefore, these traditional sources are still being searched for new microorganisms.
2. Genetic manipulation of microorganisms
 - a. Mutation - once a promising culture is found, it can be improved by mutagenesis with chemical agents and UV light
 - b. Protoplast fusion - Widely used with yeasts and molds, especially if the microorganism is asexual or of a single mating type; involves removal of cell walls, mixing two different solutions of protoplasts, and growth in selective media.
 - c. Site-directed mutagenesis is used to insert short lengths of DNA into specific sites in genome of a microorganism; leads to small changes in amino acid sequence, but these can result in unexpected changes in protein characteristics; site-directed mutagenesis is important to field of protein engineering
3. Microbial products are often classified as primary or secondary metabolites
 - a. Primary metabolites are related to the synthesis of microbial cells in the growth phase; they include amino acids, nucleotides, fermentation end products, and exoenzymes
 - b. Secondary metabolites usually accumulate in the period of nutrient limitation or waste product accumulation that follows active growth; they include antibiotics and mycotoxins

Major Products of Industrial Microbiology

Antibiotics

Penicillin-careful adjustment of medium composition is used to slow growth and to stimulate penicillin production; side chain precursors can be added to stimulate production of particular penicillin derivatives; harvested product can then be modified chemically to produce a variety of semisynthetic penicillins

Streptomycin is a secondary metabolite that is produced after microorganism growth has slowed due to nitrogen limitation

Amino acids

Amino acids such as lysine and glutamic acid are used as nutritional supplements and as flavor enhancers

Amino acid production is usually increased through the use of regulatory mutants or through the use of mutants that alter pathway architecture

Organic acids

These include citric, acetic, lactic, fumaric, and gluconic acids

Citric acid, which is used in large quantities by the food and beverage industry, is produced largely by *Aspergillus niger* fermentation in which trace metals are limited to regulate glycolysis and the TCA cycle, thereby producing excess citric acid

Gluconic acid is also produced in large quantities by *A. niger*, but only under conditions of nitrogen limitation; gluconic acid is used in detergents

Specialty compounds for use in medicine and health-include sex hormones, ionophores, and compounds that influence bacteria, fungi, amoebae, insects, and plants

Biopolymers (microbially produced polymers): Polysaccharides are used as stabilizers, agents for dispersing particulates, and as film-forming agents; they also can be used to maintain texture in ice cream, as blood expanders and absorbents, to make plastics, and as food thickeners; also used to enhance oil recovery from drilling mud

Cyclodextrins can modify the solubility of pharmaceuticals, reduce their bitterness, and mask their chemical odors; can also be used to selectively remove cholesterol from eggs and butter and protect spices from oxidation

Biosurfactants

Biosurfactants may replace chemically synthesized surfactants because of increased biodegradability, which thereby creates better safety for environmental applications

The most widely used biosurfactants are glycolipids, which are excellent dispersing agents

Bioconversion processes (microbial transformations or biotransformations)

Microorganisms are used as biocatalysts; bioconversions are frequently used to produce the appropriate stereoisomer, are very specific, and can be carried out under mild conditions

When bioconversion reactions require ATP or reductants, an energy source must be supplied.

Microbial Enzymes and Their Uses – Microbial enzymes are also used for production of synthetic polymers. Plastic industry mostly uses chemical methods for producing alkene oxides used in the production of plastics. It is now possible to synthesise alkene oxides by using microbial enzymes and genetically engineered strains would make commercial production feasible.

The synthesis of alkene oxides from alkenes requires sequential action of three enzymes: pyranose-2-oxidase from the fungus *Oudemansiella mucida*, a haloperoxidase from the fungus *Caldariomyces*, and an epoxidase from a *Flavobacterium* sp.

Some important **Products of Industrial Microbiology** are tabulated on the next page.

Microorganism	Final product
Industrial 'oxychemicals' (alcohols & solvents) <i>Saccharomyces cerevisiae</i> <i>Kluyveromyces fragilis</i> <i>Clostridium acetobutylicum</i> <i>Saccharomyces</i> sp. <i>Acetobacter</i> sp. <i>Bacillus</i> sp.	Ethanol Ethanol Acetone, isopropanol & butanol Glycerol Sorbitol Propylene glycol
Organic acids <i>Aspergillus niger</i> <i>Lactobacillus delbrueckii</i> <i>Bacillus</i> sp. <i>Acetobacter</i> sp. <i>Propionibacterium shermanii</i> <i>Rhizopus</i> sp.	citric acid Lactic acid Acrylic acid Acetic acid Propionic acid Fumaric acid
Enzymes <i>Aspergillus niger</i> / <i>A. oryzae</i> <i>Bacillus subtilis</i> <i>Trichoderma reesei</i> <i>Saccharomyces cerevisiae</i> <i>S. lipolytica</i> <i>Aspergillus</i> spp. / <i>Rhizopus oryzae</i> <i>Saccharomyces lactis</i> / <i>Rhizopus oryzae</i> <i>Bacillus licheniformis</i> <i>Bacillus coagulans</i>	Glucoamylase Amylase/ neutral protease cellulase Invertase Lipase Pectinases Lactase Alkaline protease Glucose isomerase
Amino acids <i>Corynebacterium glutamicum</i> <i>Brevibacterium</i> spp.	L-lysine Glutamic acid
Vitamins <i>Ashbya gossypii</i> <i>Pseudomonas denitrificans</i> <i>Propionibacterium shermanii</i>	Riboflavin Vitamin B12 Vitamin B12
Polysaccharides <i>Leuconostoc mesenteroides</i> <i>Xanthomonas campestris</i>	Dextran Xanthan gum
Bioinsecticides <i>Bacillus thuringiensis</i> <i>Bacillus popilliae</i>	Bt-toxin (anti-insect larval compd.) Control of mosquitoes
Food supplements Methanogenic bacteria <i>Spirulina</i> sp. / <i>Fusarium</i> sp. <i>Rhizopus oryzae</i>	Single cell protein (SCP) SCP Single cell oil (SCO)
Pharmaceuticals (Antibiotics) <i>Penicillium chrysogenum</i> <i>Cephalosporium acremonium</i> <i>Streptomyces</i> spp.	Penicillin and it relatives Cephalosporins Streptomycin, Neomycins, Tetracyclines, Amphoterecin-B Kanamycins, Polyoxins, Actidione Gramicidin-S Polymixin-B
<i>Bacillus brevis</i> <i>Bacillus polymyxa</i>	
Pharmaceuticals (other than antibiotics) <i>Rhizopus nigricans</i> <i>Escherichia coli</i> (by recombinant DNA technol.)	Steroids (by transformations) Alpha-1 antitrypsin (against <i>amphysema</i> or lung distension) Insulin (hormone for diabetes) Interleukins (antitumour)

Bioremediation – Applications of microbes in control of soil and water pollution

The need for bioremediation

Enormous quantities of organic and inorganic compounds are released into the environment each year because of human activities. In some cases these releases are deliberate and well regulated (e.g. industrial emissions) while in other cases they are accidental (e.g. chemical or oil spills). Petroleum and its products are one of the most common environmental pollutants. They are a fire hazard, threat to marine life, and a source of air and groundwater pollution. They contaminate land and water bodies by accidental spills like the Alaska Oil spill in 1989 and oil spills during the Gulf War, leakage from pipelines, and other human activities. Detoxification of the contaminated sites is expensive and time consuming by conventional chemical or physical methods. In this context, Bioremediation emerges as an alternative technique that is not only efficient and environment friendly, but also highly cost-effective.

What is bioremediation?

Bioremediation can be defined as any process that uses microorganisms, fungi, green plants or their enzymes to return the natural environment altered by contaminants to its original condition. Bioremediation consists of using naturally occurring or laboratory cultivated microorganisms to reduce or eliminate toxic pollutants. Bioremediation technology using microorganisms was reportedly invented by George M. Robinson in 1960s, when he was the assistant county petroleum engineer for Santa Maria, California, where he experimented with microbes in cleaning of polluted sites.

Approaches to bioremediation

Bioremediation technologies can be generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the contaminated material at the site while *ex situ* involves the removal of the contaminated material to be treated elsewhere. Some examples of bioremediation technologies are as follows:

1. **Biostimulation:** Nutrients and oxygen – in a liquid or gas form – are added to contaminated water or soil to encourage the growth and activity of bacteria already existing in the soil or water. The disappearance of contaminants is monitored to ensure that remediation occurs.
2. **Bioaugmentation:** Microorganisms that can clean up a particular contaminant are added to the contaminated soil or water. Bioaugmentation is more commonly and successfully used on contaminants removed from the original site, such as in municipal wastewater treatment facilities. To date, this method has not been very successful when done at the site of the contamination because it is difficult to control site conditions for the optimal growth of the microorganisms added.
3. **Intrinsic Bioremediation:** Also known as natural attenuation, this type of bioremediation occurs naturally in contaminated soil or water. This natural bioremediation is the work of microorganisms and is seen in petroleum contamination sites, such as old gas stations with leaky underground oil tanks. Researchers are studying whether intrinsic bioremediation happens in areas with other types of chemical contamination. Application of this technique requires close monitoring of contaminant degradation to ensure that environmental and human health are protected.
4. **Mycoremediation:** Mycoremediation is a form of bioremediation, the process of using mushrooms to return an environment (usually soil) contaminated by pollutants to a less contaminated state. The term *mycoremediation* was coined by Paul Stamets and refers specifically to the use of fungal mycelia in bioremediation.

One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium. The mycelium secretes extracellular enzymes and acids that break down lignin and cellulose, the two main building blocks of plant fiber. These are organic compounds composed of long chains of carbon and hydrogen, structurally similar to many organic pollutants.

The key to mycoremediation is determining the right fungal species to target a specific pollutant. Certain strains have been reported to successfully degrade the nerve gases VX and sarin.

In an experiment conducted in conjunction with Thomas, a major contributor in the bioremediation industry, a plot of soil contaminated with diesel oil was inoculated with mycelia of oyster mushrooms; traditional bioremediation techniques (bacteria) were used on control plots. After four weeks, more than 95% of many of the PAH (polycyclic aromatic hydrocarbons) had been reduced to non-toxic components in the mycelial-inoculated plots. It appears that the natural microbial community participates with the fungi to break down contaminants, eventually into carbon dioxide and water. Wood-degrading fungi are particularly effective in breaking down aromatic pollutants (toxic components of petroleum), as well as chlorinated compounds (certain persistent pesticides)

Scientific principles in bioremediation

Bioremediation depends on the natural biological processes of microorganisms, one of which is metabolism. Metabolism refers to all the chemical reactions that happen in a cell or organism. All living processes are based on a complex series of chemical reactions. Metabolic processes fall into two types – those that build complex molecular structures from simpler molecules, called anabolism, and those that break down complex molecules into simpler molecules, called catabolism. Chemicals present in contaminated sites can be remediated through either, or both, of these processes.

Chemicals present at contaminated sites become part of the anabolism and catabolism process. For example, hydrocarbons (part of the carbon family) present at sites with petroleum products can be taken up by microorganisms and used as building blocks for cell components.

Other chemicals that are important to a microorganism include chemical compounds in the phosphorus, potassium, calcium and sodium group. Microorganisms also need trace elements of other chemicals, including chromium, cobalt, copper, and iron, all of which can be available in abundance at contaminated sites.

Advantages of bioremediation

There are a number of cost and efficiency advantages to bioremediation, which can be employed in areas that are inaccessible without excavation. For example, hydrocarbon spills (specifically, petrol spills) or certain chlorinated solvents may contaminate groundwater, and introducing the appropriate electron acceptor or electron donor amendment, as appropriate, may significantly reduce contaminant concentrations after a lag time allowing for acclimation. This is typically much less expensive than excavation followed by disposal elsewhere, incineration or other ex situ treatment strategies, and reduces or eliminates the need for "pump and treat", a common practice at sites where hydrocarbons have contaminated clean groundwater.

In the recent years, various microorganisms are being studied to see if they can remediate various chemicals often present at contaminated industrial sites. In addition, scientists are currently looking into genetically engineering certain microorganisms to increase their ability to metabolize specific chemicals, such as hydrocarbons, in contaminated sites.

Microbiological Wastewater Treatment

Many wastewater treatment technologies are dependent on beneficial microorganisms for remediation of wastewater. One of the primary goals of biological treatment is the removal of organic material from wastewater so that excessive oxygen consumption does not become a problem when it is released to the environment.

Another goal of biological treatment is nitrification / denitrification. Nitrification is an aerobic process in which bacteria oxidize reduced forms of nitrogen. Denitrification is an anaerobic process by which oxidized forms of nitrogen are reduced to gaseous forms, which can then escape into the atmosphere. This is important because the release of nitrogen to the aquatic environment can also cause eutrophication.

Another goal of biological treatment is elimination of pathogenic microorganisms either through predation or out-competition. The oxidation/stabilization of organic sludge is also of importance in biological treatment of wastewater.

The basic mechanisms of biological treatment are the same for all treatment processes. Microorganisms, principally bacteria, metabolize organic material and inorganic ions present in wastewater during growth.

PART – II

FUNGI

Introduction to Fungi

An overview

The term **fungi** is defined in two ways in modern botanical literature.

1. **In non-taxonomic way:** According to this explanation, the fungi are heterotrophic eukaryotes, showing sporulative multiplication, filamentous growth and absorptive mode of nutrient acquisition (osmotrophy).
2. **In a taxonomic sense:** This explanation attempts at creating a phylogenetically cohesive group. According to this, the fungi are heterotrophic eukaryotes having originated from the protistan group Choanoflagellata, showing chitinous cell wall, filamentous growth (if not unicellular), sporulative multiplication and absorptive mode of nutrient acquisition (osmotrophy).

The above taxonomic definition of fungi applies only to the members of the Kingdom Mycota within the domain Eukarya. It is noteworthy that the Kingdom Mycota, as it is currently organized, does not include the Oomycetes and the Myxomycetes. In this kingdom, there are seven phyla considered currently (Hibbett, D.S., et al. 2007).

1. Phylum Chytridiomycota
2. Phylum Blastocladiomycota
3. Phylum Neocallimastigomycota
4. Phylum Zygomycota
5. Phylum Glomeromycota
6. Phylum Ascomycota
7. Phylum Basidiomycota

Because of some similarities in morphology and lifecycle, Myxomycetes and Oomycetes were formerly classified in the kingdom Fungi. Molecular evidences reveal that neither water molds (Oomycetes) nor slime molds (Myxomycetes) are closely related to the true fungi, and, therefore, taxonomists no longer group them in the kingdom Fungi. Now slime molds are grouped in the Amoebozoa and water molds are grouped in the Stramenopila kingdom. These organisms are now treated as **Fungi-like organisms**, rather than true Fungi.

The salient features of the Fungi include the following:

1. Occurring worldwide especially under somewhat humid conditions and living for the most part in soil, dead matter, and as symbionts of plants, animals, or other fungi.
2. Largely invisible to the unaided eye due to very small size
3. Eukaryotic organization of cells
4. A chitinous cell wall
5. Majority of species grow as multicellular / multinucleate filaments called hyphae forming a mycelium; some fungal species also grow as single cells.
6. Heterotrophic behaviour with absorptive mode of food acquisition.
7. All the phyla show monoploid genetic constitution in the somatic stage.
8. Asexual reproduction of the fungi is commonly via spores
9. Sexual reproduction is mediated by gametic fusion, gametangial fusion, spermatization or even somatogamy. Sexual reproduction with meiosis exists in all fungal phyla, except the Deuteromycota.
10. Many fungal species have elaborate vegetative incompatibility systems that allow mating only between individuals of opposite mating type, while others can mate and sexually reproduce with any other individual or itself. Species of the former mating system are called heterothallic, and of the latter homothallic.
11. Some species have lost the ability to form reproductive structures, and propagate solely by vegetative growth.
12. Fungi produce several secondary metabolites functioning as defensive compounds or for niche adaptation. Many such products are of commercial interest.

How are the fungi different from the plants?

For a long time taxonomists considered fungi to be members of the Plant Kingdom. This early classification was based mainly on similarities in lifestyle, for example:

1. both fungi and plant are mainly sessile
2. both have similarities in general morphology and growth habitat (like plants, fungi often grow in soil, in the case of mushrooms forming conspicuous fruiting bodies, which sometimes bear resemblance to plants such as mosses)
3. both groups possess a cell wall, which is absent in the Animal Kingdom
4. the fungi reproduce by spores, which other cryptogams also do.

However, the fungi are now considered a separate kingdom, distinct from both plants and animals. It is now accepted that the true fungi arose about 1,200 million years ago from the protistan group Choanoflagellata, the same group that also gave rise to the metazoans.

Many studies have identified several distinct morphological, biochemical, and genetic features in the Fungi, clearly delineating this group from the other kingdoms. For the reasons mentioned below, the fungi are placed in their own kingdom.

1. Similar to animals and unlike most plants, fungi lack the capacity to synthesize organic carbon by chlorophyll-based photosynthesis.
2. Plants store the reduced carbon as starch, fungi, like animals and some bacteria, use glycogen for storage of carbohydrates.
3. A major component of the cell wall in many fungal species is the nitrogen-containing carbohydrate, chitin, also present in some animals, such as the insects and crustaceans, while the plant cell wall consists chiefly of the carbohydrate cellulose.
4. The defining and unique characteristics of fungal cells include growth as hyphae, which are microscopic filaments of between 2-10 microns in diameter and up to several centimetres in length, and which combined form the fungal mycelium. Some fungi, such as yeasts, grow as single ovoid cells, similar to unicellular algae and the protists. Such types of somatic organization are totally absent in plants.
5. Fungi show heterotrophy by absorption, which is nearly absent in plants.
6. Unlike many plants, most fungi lack a vascular system, such as xylem or phloem for long-distance transport of water and nutrients; as an example for convergent evolution, some fungi, such as *Armillaria*, form rhizomorphs or mycelial cords, resembling and functionally related to, but morphologically distinct from, plant roots.
7. The fungi for most of the part in their life cycle remain monoploid while all the plants essentially show alternation of generation type of life cycle.
8. A further characteristic of the fungi that is absent from other eukaryotes, and shared only with some bacteria, is the biosynthesis of the amino acid, L-lysine, via the α -aminoadipate pathway.
9. Although plants and fungi have a similar pathway in the biosynthesis of terpenes using mevalonic acid and pyrophosphate as biochemical precursors; plants also use an additional terpene biosynthesis pathway in the chloroplasts that is apparently absent in fungi.
10. 18S rRNA sequence comparison also places the Fungi closer to the animals.

There can be no doubt that the fungi are more closely related to animals than plants, yet the discipline of biology devoted to the study of fungi, known as Mycology, often falls under a branch of botany as a matter of tradition till date.

The major groups of Fungi

1. The **Chytridiomycota** are commonly known as chytrids. Chytrids produce zoospores that are capable of active movement through aqueous phases with a single flagellum.
2. The **Blastocladiomycota** were previously considered a taxonomic clade within the Chytridiomycota. Recent molecular data and ultrastructural characteristics, however, place the Blastocladiomycota as a sister clade to the Zygomycota, Glomeromycota, and Dikarya (Ascomycota and Basidiomycota). The blastocladiomycetes are fungi that are saprotrophs and parasites of all eukaryotic groups and undergo sporic meiosis unlike their close relatives, the chytrids, which mostly exhibit zygotic meiosis.
3. The **Neocallimastigomycota** were earlier placed in the phylum Chytridiomycota. Members of this small phylum are anaerobic organisms, living in the digestive system of larger herbivorous mammals and possibly in other terrestrial and aquatic environments. They lack mitochondria but

contain hydrogenosomes of mitochondrial origin. As the related chytrids, neocallimastigomycetes form zoospores that are posteriorly uniflagellate or polyflagellate.

4. The **Zygomycota** contain the taxa, Zygomycetes and Trichomycetes, and reproduce sexually with meiospores called zygospores and asexually with sporangiospores. Black bread mold (*Rhizopus stolonifer*) is a common species that belongs to this group; another is *Pilobolus*, which is capable of ejecting spores several meters through the air. Medically relevant genera include *Mucor*, *Rhizomucor*, and *Rhizopus*. Molecular phylogenetic investigation has shown the Zygomycota to be a polyphyletic phylum with evidence of paraphyly within this taxonomic group.
5. Members of the **Glomeromycota** were once considered within Zygomycota. They are fungi forming arbuscular mycorrhizae with higher plants. Only one species has been observed forming zygospores; all other species solely reproduce asexually. The symbiotic association between the Glomeromycota and plants is ancient, with evidence dating to 400 million years ago.
6. The **Ascomycota**, commonly known as sac fungi or ascomycetes, constitute the largest taxonomic group within the Eumycota. These fungi form meiotic spores called ascospores, which are enclosed in a special sac-like structure called an ascus. This division includes morels, a few mushrooms and truffles, single-celled yeasts (e.g., of the genera *Saccharomyces*, *Kluyveromyces*, *Pichia*, and *Candida*), and many filamentous fungi living as saprotrophs, parasites, and mutualistic symbionts. Prominent and important genera of filamentous ascomycetes include *Aspergillus*, *Penicillium*, *Fusarium*, and *Claviceps*. Many ascomycetes species have only been observed undergoing asexual reproduction (called anamorphic species), but molecular data has often been able to identify their closest teleomorphs in the Ascomycota. Because the products of meiosis are retained within the sac-like ascus, several ascomycetes have been used for elucidating principles of genetics and heredity (e.g. *Neurospora crassa*).
7. Members of the **Basidiomycota**, commonly known as the club fungi or basidiomycetes, produce meiospores called basidiospores on club-like stalks called basidia. Most common mushrooms belong to this group, as well as rust (fungus) and smut fungi, which are major pathogens of grains. Other important Basidiomycetes include the maize pathogen, *Ustilago maydis*, human commensal species of the genus *Malassezia*, and the opportunistic human pathogen, *Cryptococcus neoformans*.

The importance of fungi

1. Fungi are the most important causes of crop diseases, responsible for massive crop damage each year, and for periodic devastating disease epidemics.
2. Fungi are the main decomposers and recyclers of organic matter, including the degradation of cellulose and wood by the specialized enzyme systems unique to fungi.
3. Fungi produce some of the most toxic known metabolites, including the carcinogenic aflatoxins and other mycotoxins in human foods and animal feedstuffs.
4. With the advance of the acquired immune deficiency syndrome (AIDS) and the increasing role of transplant surgery, fungi are becoming one of the most significant causes of death of immune-compromised and immune-suppressed patients. Fungal diseases that were once extremely rare are now commonplace in this sector of the population.
5. Fungi have an enormous range of biochemical activities that are exploited commercially – notably the production of antibiotics (e.g. penicillins), steroids (for contraceptives), cyclosporins (used as immunosuppressants in transplant surgery), and enzymes for food processing and for the soft drinks industry.
6. Fungi are major sources of food. They are used for bread-making, for mushroom production, in several traditional fermented foods, for the production of mycoprotein – a type of “single-cell protein” ventures of the late 1900s – and for the production of alcoholic drinks.
7. Fungi can be used as “cellular factories” for producing heterologous (foreign) gene products. The first genetically engineered vaccine approved for human use was produced by engineering the gene for hepatitis B surface antigen into the yeast (*Saccharomyces cerevisiae*) genome. In this way the antigen can be produced and exported from the cells, then purified from the growth medium.
8. The genome sequences of several fungi have now been determined, and in several cases the genes of fungi are found to be homologous (equivalent) to the genes of humans. So, fungi can be used to investigate many fundamental cell-biological processes, including the control of cell division and differentiation relevant to biomedical research.
9. Fungi are increasingly being used as commercial biological control agents, providing alternatives to chemical pesticides for combating insect pests, nematodes, and plant-pathogenic fungi.

Economic aspects of fungi

Fungi as Food

- Fungi have been used as food since times immemorial and esteemed as delicacies. The fructifications of several mushrooms and morels (e.g. *Agaricus bisporus*, *Amanita vaginata*, *Boletus edulis*, *Clavatia gigantea*, *Lentinus edodes*, *Morchella*, *Pleurotus*, *Volvaria*, *Volvariella volvacea*) are used as food. *Agaricus campestris* is cultivated in many areas of north and south India and *Morchella esculenta* is grown in Punjab and Kashmir. Mushrooms are preferred for food because of their large protein contents, vitamins, carbohydrates, minerals and amino acids.
- Role of a leavening agent for bread-making. Eg. – Baker's strain of the yeast *Saccharomyces cerevisiae*
- Fermentation to produce alcoholic beverages – such as Wines and Champagnes, Beers and Ales, Distilled spirits. Strains of *Saccharomyces cerevisiae* or *S. Ellipsoideus* – used in wine preparation. Most beers are fermented with bottom yeasts, related to *Saccharomyces pastorianus*. Bottom yeasts produce beer with a pH of 4.1 to 4.2 and requiring 7-12 days of fermentation.
- Yeast is an important source of vitamin B and D. *Saccharomyces*, *Endomyces*, *Rhodotorula*, and *Torulopsis* are particularly rich in proteins and hence they are mixed in incomplete livestock.
- A popular food – **sufu** – is produced from species of *Mucor* and *Antimucor*.
- Some foods like soybeans and cassava although rich in nutrients, cannot be easily digested by man but can be made palatable by fermenting fungi. Soybeans are fermented by species of *Rhizopus* (eg. *R. oligosporus*, *R. oryzae*) to prepare **tempeh**, a food more digestible and tasty.
- Similarly, **Incaparina** (protein cakes) is prepared by mixing yeasts or *R. oligosporus* with some cereal flours to increase protein contents. Such cakes are rich in niacin and riboflavin.
- Yeasts grown on ammonia rich molasses yield **food yeast** that has 40-50% proteins and vitamin B complex. This large-scale production of yeasts as food is called microbial farming.
- Preparation of jalebies, Idli, Kanji, warries, etc. involve fermentation by *Saccharomyces*, *Torulopsis*, *Trichosporon*, and *Hansenula anomala*.
- SCP** (Single Cell Protein) is a microbial protein obtained from algae, fungi, yeast and bacteria. Fungi, like *Fusarium*, *Aspergillus*, *Penicillium*, *Rhizopus*, and yeast like *Candida*, *Torulopsis*, are exploited for the production of SCP. Fungi contain 19-47% protein contents and are rich in methionine, Vitamin B₁₂ and riboflavin. Yeasts contain 45-55% more digestible form of protein and are rich in vitamins most of essential amino acids except methionine. They also have salts and fats.

Role of Fungi in Medicine

Several fungal members are used in the production of antibiotics, proteins, vitamins, ergot, steroids and other useful products.

- Calvatia gigantea* (giant puff ball) yields Calvacin having anti-cancer properties. It's eating prevents stomach tumors.
- Vitamins – Yeasts are good source of vitamin B complex, and D. *Torulopsis utilis* and *Rhodotorula rubra* are rich in proteins, fats and vitamins of B complex like niacin, folic acid and thiamine and called food yeast or vitaminised food. It has 15% proteins. Ergosterol (a precursor of vitamin D) is synthesized from some moulds and yeast. *Eremothemium ashbyii* is a rich source of Vitamin B₁₂, whereas vitamin A is extracted from *Rhodotorula gracilis*.
- Ergot – Ergot is prepared from the sclerotia of *Claviceps purpurea*. It contains some alkaloids which are used to induce uterine contraction for abortion, in the treatment of menstrual disorders and to check haemorrhages. However, excess of ergot leads to serious convulsions or a gangrenous condition that may result in loss of limbs.
- Ephedrine – It is synthesized from benzaldehyde by the action of yeast and is used in the treatment of asthma and nasal troubles.
- Steroids – Steroids are complex organic compounds, effective against rheumatoid arthritis, allergic, dermatologic and other diseases. They are also used as anaesthetic and antifertility agents. Steroids are adrenal cortical and gonadal hormones and their derivatives. Their extraction from biological system is highly expensive. A wide variety of fungi has the capacity of synthesizing many steroids; for instance, cortisone is prepared by the fermentation of plant glycosides by *Rhizopus nigricans* and *Aspergillus niger*.

- Antibiotics – Antibiotics are metabolic substances produced by some living organisms which are injurious to other living beings. Alexander Fleming (1944) extracted the wonder drug **penicillin** from *Penicillium notatum*. Since then several other antibiotic have been extracted from fungi. Eg –

	Antibiotics	Source	Range of action
1	Proliferin	<i>Aspergillus proliferans</i>	Bacteria
2	Ramycin	<i>Mucor remannianus</i>	Bacteria
3	Ustilagic acid	<i>Ustilago maydis</i>	Fungi
4	Fumagillin	<i>Aspergillus fumigates</i>	Amoeba, Staphylococcal bacteriophage
5	Trichothecin	<i>Trichothecium roseum</i>	Virus, fungi

Several fungi are active against human pathogens; for instance spread of *Candida albicans* is prevented by the extract of *Tricholoma saponaceum*. Mushrooms like *Agaricus bisporus*, possess antitumour properties. Many edible mushrooms have the ability to lower blood cholesterol.

Some examples:

- Bio-dyne is derived from yeast cells. It is used in the healing and repairing of tissues.
- Fungi as Test Organisms (Biological Assays) – Living fungi are used to assay potency of drugs and to detect and estimate some chemicals. *Aspergillus niger* strains are used to detect trace elements like Zn, Cu, Pb, and Mo in the substrate. These elements when taken up by the fungus may give a particular color to the conidia. *Neurospora crassa* is used as test organism to detect presence of vitamin B complex in a sample. Auxotrophs (Nutritional mutants) of *Neurospora* are used for amino acid assays.
- Fungi in Research – *Neurospora*, yeast, *Sordaria*, *Glomerella*, *Ascobolus* – are widely used as research material.

Role of Fungi in Industry

- In brewery** – Alcoholic fermentation with the help of fungi is the basis of brewing industries. Wine is produced by fermenting rice with *Aspergillus oryzae*. This method is popular in Japan and many European countries. *Saccharomyces cerevisiae* is used in the production of beer.
- In baking industry** – Fermentation of carbohydrates by *Saccharomyces cerevisiae* produces ethyl alcohol and CO₂.

$$C_6H_{12}O_6 \rightarrow (\text{in presence of yeast}) \rightarrow 2C_2H_5OH + 2CO_2$$
Carbon dioxide liberated in this process is used in the preparation of several bakery products such as breads and cakes.
- In cheese industry** – Some species of *Penicillium* (e.g. – *P. camembertile*, *P. roqueforti*, *P. candidum*) are used for maturation of cheese.
- Fungi in Enzyme production** – Many intra and extra-cellular enzymes are found in fungi and some are extracted on commercial scale. Eg –
 - Invertase** – The yeast *Saccharomyces cerevisiae* is used for extraction of the enzyme invertase. This enzyme shydrolyses sugars into glucose and fructose and is used in confectionary and paper industry.
 - Zymase** – Enzyme zymase also obtained from *Saccharomyces cerevisiae* and used in the preparation of ethyl alcohol by fermentation of carbohydrates.
 - Amylase** – *Aspergillus niger* and *A. oryzae* are used to produce amylase – used in alcohol industry, in the manufacture of dextrinized starch and in medicines.
 - Cellulase** – *Trichoderma reesli* is used in production of cellulose – used in production of cheese and in saccharification of cellulosic and lignocellulosic wastes together with recycling of hydrosylate for production of SCP, microbial biomass, vitamins, etc.
 - Lipases, pectnases, glucose oxidase, proteases and lactase** – also obtained from fungi.
- Fungi in production of Organic Acids** – Biochemical activities of several fungi are utilised in the commercial production of organic acids such as,
 - Citric acid** – produced by fermenting sucrose and molasses by *Aspergillus niger* and *A. wentii*. Citric acid is used in soft drinks and other foods and medicinal preparations. It is a superior sequestering agent and is used in the manufacture of ink, dyeing, electroplating and leather tanning.

2. Fumaric acid – is obtained by fermentation of sugars by *Rhizopus stolonifer*. It is used in the manufacture of alkyl resins and wetting agents.
3. Itaconic acid, gluconic acid, kojic acid, gallic acid, lactic, oxalic and succinic acids – all are produced by fungi.
- **Lipids** – produced by the activity of *A. niger*, *Rhizopus*, etc.
- **Pigments** – Some fungal spores are brightly colored and used as dyestuffs or coloring food articles. Spinulesin is a blue pigment obtained from *P. spinulosus*; catenarin (red pigment) from *Helminthosporium*.
- **Litmus paper** and some perfumes are made from some lichens.
- *Oidium lactis* is used in plastic industry.
- **Gibberellins** is obtained from *Fusarium moniliforme* and is used in flowering, parthenocarpy, germination and growth.

Fungi in Agriculture

- **As scavengers** – Saprophytic fungi and bacteria decompose the dead matter and excreta of organisms and thereby act as scavengers. *Chaetomium globosum* is called vegetable vulture and helps in recovering the soil nutrients. During decomposition, large amounts of CO₂ is evolved and minerals and humus are added to the soil. In the absence of these scavengers, the surface of the earth would have covered with remains of dead animals and plants.
- **Soil aggregation and Soil fertility** – Some fungi, such as species of *Absidia*, *Aspergillus*, *Cladosporium*, *Mucor*, *Penicillium*, *Rhizopus*, etc., have soil-binding properties. The mucilaginous substances secreted by them help in soil aggregation. Yeast such as *Rhodotorula*, and *Saccharomyces* and several other phylloplane fungi have nitrogen-fixing capabilities, thus increasing soil fertility. In a forest ecosystem, the natural mushroom flora greatly helps in biodegradation of woody wastes. The ultimate end product in the form of humus is quite useful for the growth of other plants.
- **Importance as mycorrhiza** – Several fungi, like species of *Rhizoctonia*, *Phoma*, *Tricholoma*, *Boletus*, *Phallus*, *Scleroderma* and *Amanita*, form mycorrhizal relationships with higher plants providing water and minerals and obtaining organic food in this symbiotic relationship.
- **In biological control** – Fungi play an important role in biological control of diseases; for instance, *Trichoderma lignorum* suppresses the growth of root rot fungus, *Pythium* and the growth of *Rhizoctonia solani* can be checked by *Penicillium vermiculatum* and *Rhizoctonia oryzae*. Fungal pathogens play an important role in nature in the reduction of weeds. The strategy of using fungal plant pathogens in biological control involves a classic tactic and a bioherbicide tactic. The first approach involves introduction of a foreign plant pathogen, while the latter envisages the development of endemic pathogens and using them as microbial weed killers (bioherbicide). Several fungi are also utilised for controlling soil-borne organisms like amoeba and nematodes. For instance, *Nematophthora gyrophila* is capable to control effectively *Heterodera avenae*, a cereal cyst nematode.
- **As growth hormones** – Gibberellin, produced by *Gibberella fujikuroi*, is an important plant hormone – accelerates growth of many crops. The hormone trisporic acid is obtained from *Mucor mucedo* and *Choanephora trispora*.
- **As insecticides** – many insect pests can be controlled by the use of fungi *Aschersonia aleyroidis*, *Fusarium oxysporium* etc.
- **Antiviral property** – The inhibition of plant viruses by fungal growth products of *Aspergillus*, *Rhizoctonia*, *Fusarium*, etc has been reported.

Systematics of fungi

Overview

As earlier noted, in current literature of Botany, a difference is made between **true fungi** and **fungi like organisms**. They are described as follows.

The term **fungi** is defined in two ways in modern botanical literature.

1. **True fungi:** They are heterotrophic eukaryotes having originated from the protistan group Choanoflagellata, showing chitinous cell wall, filamentous growth (if not unicellular), sporulative multiplication and absorptive mode of nutrient acquisition (osmotrophy). These organisms are placed in Kingdom Mycota (also called Eumycota). Important phyla of true fungi are:
 - a. Phylum Chytridiomycota
 - b. Phylum Zygomycota
 - c. Phylum Ascomycota
 - d. Phylum Basidiomycota
2. **Fungi like organisms:** These organisms have a lot of structural, functional and reproductive features similar to the true fungi but they do not have chitin wall. Further, molecular phylogenetic evidences show that they have diverse origins rather than descent from the protistan group Choanoflagellata (the ancestor of the true fungi). Important phyla of fungi like organisms are:
 - a. Phylum Myxomycota
 - b. Phylum Hyphochytridiomycota
 - c. Phylum Plasmodiophoromycota
 - d. Phylum Oomycota

Classification adopted by modern literature

Fungi in the widest sense, as organisms traditionally studied by mycologists, currently fall into three kingdoms of Eukaryota, i.e.

1. **Eumycota** which contain only true fungi
2. **Protozoa** which contain some fungi like organisms
3. **Chromista (= Straminipila)** which contain mainly fungi like organisms

Webster (2007) has adopted the following scheme in his book *An Introduction to Mycology*.

Kingdom Protista

The Protists are diverse and ancient single-celled organisms, which have descended from a unicellular ancestor along separate lines. It includes slime moulds, which do not form hyphae, lack cell walls in the somatic phase, and also ingest nutrients in particulate form by phagocytosis. Therefore they do not fit into definition of fungi except for the production of fruiting bodies apparently similar to those of fungi. Since they grow in similar habitats as fungi, they attracted the attention of mycologists who studied them and thus included them in the textbooks of fungi. Two of the thoroughly studied groups of slime moulds by mycologists are:

1. The cellular slime moulds (**Acrasiomycetes** and **Dictyosteliomycetes**)
2. The plasmodial slime moulds (**Myxomycetes**).
3. Another group of fungi included in protozoa are plasmodiophorids, (**Plasmodiophoromycota**) which are obligate intracellular parasites of fungi, algae and higher plants and exist as naked plasmodia in the host cells.

FUNGI LIKE ORGANISMS IN PROTISTA

Phylum Myxomycota

Class Acrasiomycetes

Class Dictyosteliomycetes

Class Myxomycetes

Phylum Plasmodiophoromycota

Class Plasmodiophorales

Kingdom Straminipila (=Stramenopila)

The Stramenopila are aquatic and primarily photosynthetic organisms. They include algae such as the seaweeds, filamentous and microscopic unicellular forms, and are studied by phycologists.

The other group included in Stramenopila belongs to fungi, informally called Oomycetes or water moulds. They have now been placed in a separate phylum, **Oomycota** based on their morphological, genetic and biochemical features. They are microscopic fungi and include important plant pathogens e.g. *Phytophthora infestans* causing potato blight, *Albugo* causing white rusts and *Plasmopara* causing downy mildews. They have motile spores with two flagella with one hairy flagellum, and coenocytic hyphae with cellulosic walls (no chitin). Two other aquatic or parasitic groups of fungi with motile spores included here are the **Hyphochytriomycota** and the **Labyrinthulomycota**.

FUNGI LIKE ORGANISMS IN STRAMENOPILA

Phylum Hyphochytriomycota

Phylum Labyrinthulomycota

Class Labyrinthulomycetes

Phylum Oomycota

Order Saprolegniales

Order Pythiales

Order Peronosporales

Kingdom Fungi (Eumycota)

The Kingdom Fungi consists purely of species that are hyphal, or related to hyphal species. They are exclusively absorptive in their nutrition, and the walls contain chitin and no cellulose. The only group with motile cells (known as zoospores) is **Chytridiomycota**.

The morphological peculiarities during the sexual phase of the life cycle, serves as an important criterion in the classification of fungi. Different groups form different types of spores during their sexual phase.

The fungi forming zygospores, ascospores and basidiospores are classified as Zygomycota, Ascomycota and Basidiomycota respectively. In Ascomycota and Basidiomycota hyphae possess numerous cross-walls. They also show hyphal anastomosis where hyphae within a fungal colony may fuse with each other when come into contact. This hyphal anastomosis, if occurs frequently, can transform the radiating hyphae of a colony into a three-dimensional network.

Hyphal anastomosis may be a key factor in allowing the mycelium of some Ascomycetes and Basidiomycetes to produce large fruit bodies. However, both the features viz. presence of cross-walls and hyphal anastomoses are usually lacking in the Zygomycota and Chytridiomycota. These groups are therefore sometimes considered as 'lower fungi', in contrast to the 'higher fungi', the Ascomycota, Basidiomycota and related forms.

PHYLA OF EUMYCOTA

Phylum Chytridiomycota

Class Chytridiomycetes

Phylum Zygomycota

Class Zygomycetes

Class Trichomycetes

Phylum Ascomycota

Class Hemiascomycetes

Class Loculoascomycetes

Class Plectomycetes

Class Pyrenomycetes

Class Discomycetes

Phylum Basidiomycota

Class Homobasidiomycetes

Class Gasteromycetes

Class Heterobasidiomycetes

Class Urediniomycetes

Class Ustilaginomycetes

Traditional Classification of fungi

G.C. Ainsworth, 1973 proposed a system of fungal classification. It was exhaustive and based on the hypothesis that the fungi constitute a separate kingdom of eukaryotes.

According to this system:

FUNGI = *Eukaryotes, parasitic or mutualistic symbionts, Saprotrophs, necrotrophs or biotrophs since they are devoid of chlorophyll. Cell wall composition very variable, majority contain chitin and glucans. Reserve materials are glycogen, oil and mannitol. Some members are yeast-like but majority with thread-like filaments called hyphae, branching profusely to form the vegetative mycelium on which spores are produced, asexually or sexually, free on hyphae or enclosed in complex mycelium.*

Ainsworth recognized two Divisions in Kingdom Fungi.

1. Myxomycota
2. Eumycota

Division Myxomycota

Wall-less and quite unusual organism only included in the fungi as mostly studied by mycologists. Possess either a plasmodium, a mass of naked, multinucleate protoplasm, pseudoplasmodium, an aggregation of separate amoeboid cells. Both of a slimy consistency, hence slime moulds.

Four classes:

1. **Acrasimycetes** (Cellular slime moulds): Assimilative phase free living amoebae which aggregate to form a pseudoplasmodium before reproduction.
2. **Hydromyxomycetes** (Net Slime moulds). Spindle-shaped cells with slimy filaments that join together to form a slimy network; mostly parasitic on marine plants.
3. **Myxomycetes** (True slime moulds). Assimilative phase a true plasmodium (i.e. multinucleate protoplasmic mass), free-living, saprophytic.
4. **Plasmodiophoromycetes** (Endoparasitic slime moulds). Small plasmodia parasitic within cells of fungi, algae or higher plants.

Division Eumycota

True fungi, all with walls. Customary to recognize five sub-divisions.

Sub-division Mastigomycotina. Motile cells – zoospores present; perfect state spores oospores. Three classes.

1. **Chytridiomycetes.** Often unicellular, zoospores have single, posterior, whiplash flagellum.
2. **Hyphochytridiomycetes.** Often unicellular, zoospores have single, anterior, tinsel flagellum.
3. **Oomycetes.** Usually mycelia (aseptate), zoospores have two flagella, one directed backwards, of whiplash type and one forwards, tinsel-type.

Sub-division Zygomycotina. Usually mycelia, aseptate, asexual spores non-motile formed inside a sporangium; perfect-state spores – zygospores. Two classes:

1. **Zygomycetes.** Usually saprophytic, parasitic or predacious, mycelium, immersed in host tissue.
2. **Trichomycetes.** Often parasitic on arthropods, mycelium not immersed in host-tissue.

Sub-division Ascomycotina. Yeasts or septate mycelium, asexual spores non-motile, not formed inside sporangium; perfect state spores-ascospores, formed in an ascus, usually within a fruit body or ascocarp. Six Classes:

1. **Hemiascomycetes.** No ascocarps and ascogenous hyphae; asci naked; thallus yeast-like or mycelia.
2. **Loculoascomycetes.** Ascocarp and ascogenous hyphae present; thallus mycelial; asci bitunicate (2-walled), fruit body an ascostroma i.e., a mass of tissue with locules.
3. **Plectomycetes.** Ascocarp and ascogenous hyphae present; thallus mycelial; asci unitunicate (1-walled) evanescent i.e., breakdown at maturity, scattered within a closed fruit body-cleistothecium.
4. **Laboulbeniomyces.** Ascocarp and ascogenous hyphae present; thallus reduced; asci regularly arranged within the ascocarp (a perithegium); asci unitunicate, inoperculate; exoparasites of arthropods.
5. **Pyrenomycetes.** Aecocarp and ascogenous hyphae present; thallus reduced; asci unitunicate; regularly arranged within the ascocarp (a perithegium); asci inoperculate with apical pore or slit.
6. **Discomycetes.** Ascocarp and ascogenous hyphae present; thallus mycelial; asci unitunicate, regularly arranged in a cup-shaped ascocarp (an apothecium); asci operculate with apical pore.

Sub-division Basidiomycotina. Yeasts or septate mycelium; asexual spores absent; if present non-motile; perfect-state spores – basidiospores formed on a basidium. Three classes:

1. *Teliomycetes*. Basidiocarp lacking; teliospores (encysted probasidia) grouped in sori or scattered within the host tissue; parasitic on vascular plants.
2. *Hymenomycetes*. Basidiocarp present, basidia typically arranged in organised layer, hymenium which is completely or partly exposed at maturity; basidia septate or aseptate; basidiospores ballistospores.
3. *Gasteromycetes*. Basidiocarp present; basidia arranged in hymenium; enclosed within the basidiocarp; basidia aseptate; basidiospores statismospores.

Sub-division Deuteromycotina or Fungi Imperfecti.

Yeasts or septate mycelium; asexual spores as in Ascomycotina; perfect-state spores absent; rare or unknown. Three classes:

1. *Blastomycetes*. Budding (yeast or yeast-like) cells with or without pseudomycelium; true mycelium lacking or not well-developed.
2. *Hyphomycetes*. Mycelial; sterile or bearing asexual spores directly on hyphae or on special branches, conidiophores.
3. *Coelomycetes*. Mycelial; asexual spores formed in a flask-shaped structure – pycnidium or on a 'pad' of fungal tissue-acervulus.

An overview of fungal reproduction

Fungi constitute a kingdom of eukaryotic organisms, with heterotrophic nutrition combined with osmotrophic acquisition of food and having a chitinous cell wall with filamentous growth (multicellular hyphae forming a mycelium) in the majority of species but not in all as some fungal species also grow as single cells.

According to the modern systematics of Fungi, four phyla of *true* fungi have been recognized.

- Phylum Chytridiomycota
- Phylum Zygomycota
- Phylum Ascomycota
- Phylum Basidiomycota

Fungal Reproduction

Each of the four fungal groups is characterized by differences in their life cycles. All fungi are characterized by having a period of vegetative growth where their biomass increases. The length of time and the amount of biomass needed before sporulation can vary significantly from one species to another.

Almost all fungi reproduce by the production of spores, but a few have lost all sporing structures and are referred to as *mycelia sterilia*. Different types of spore are produced in different parts of the life cycle.

Reproduction in Chytridiomycota

Chytrids are lower fungi, quite distinct from other fungi as they have extremely simple thalli and motile zoospores. Some species within this group can be so simple that they consist of a single vegetative cell within (endobiotic) or upon (epibiotic) a host cell, the whole of which is converted into a sporangium, a structure containing spores. These types are termed holocarpic forms.

Other members of this group have a more complex morphology, and have rhizoids and a simple mycelium.

Asexual reproduction

Asexual reproduction in the chytrids is by the production of motile zoospores in sporangia that are delimited from the vegetative mycelium by complete septae. The zoospores have a single, posterior flagellum.

Sexual reproduction

Sexual reproduction occurs in the chytrids by either somatic fusion of haploid cells, or two different mating-type mycelia, or fusion of two motile gametes, or fusion of one motile gamete with a non-motile egg (Fig. 1). The production of diploid spores from the zygotic cell occurs after the sexual fusion. The resulting spore may undergo meiosis to produce a haploid mycelium or it may germinate to produce a diploid vegetative mycelium, which can undergo asexual reproduction by production of diploid zoospores. The diploid mycelium can also produce resting sporangia in which meiosis occurs, generating haploid zoospores that germinate to produce haploid vegetative mycelium.

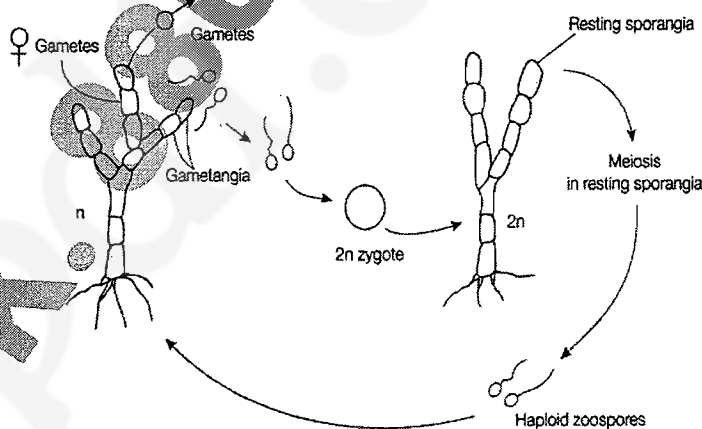


Figure 1: Reproduction in Chytridiomycota

Reproduction in Zygomycota

Asexual Reproduction

In this phylum of lower fungi, asexual reproduction begins with the production of aerial hyphae. The tip of an aerial hypha, now called a sporangiophore, is separated from the vegetative hyphae by a complete septum called a columella. The cytoplasmic contents of the tip differentiate into a sporangium containing many asexual spores. The spores contain haploid nuclei derived from repeated mitotic divisions of a nucleus from the vegetative mycelium. Dispersal of the spores is by wind or water (Fig. 2).

Sexual Reproduction

In sexual reproduction, two nuclei of different mating types fuse together within a specialized cell called a zygospore (Fig. 2). In some species the different mating-type nuclei may be within one mycelium (*homothalism*). In other species, two mycelia with different mating-type nuclei must fuse (*heterothalism*). In both cases, fusion occurs between modified hyphal tips called *progametangia*, which once fused are termed the *zygospore*.

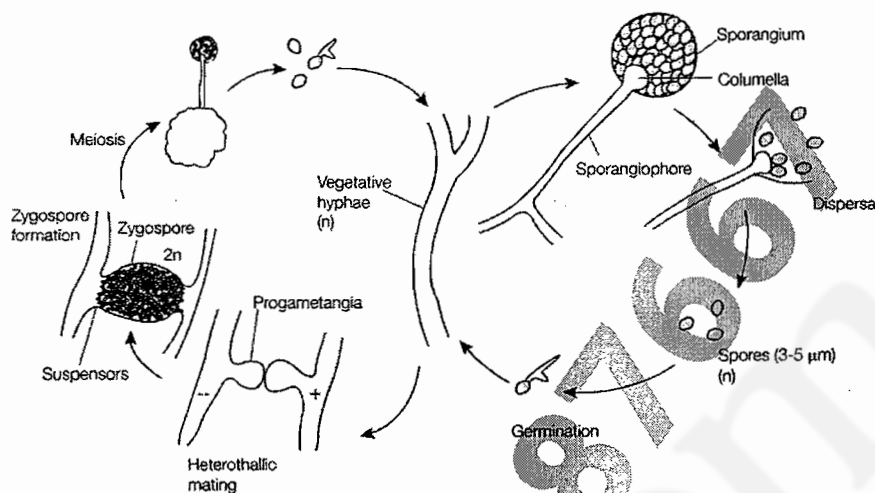


Figure 2: Reproduction in a Member of Zygomycota

Within the developing zygospore meiosis occurs; usually three of the nuclear products degenerate, leaving only one nuclear type present in the germinating mycelium.

Reproduction in Ascomycota

Asexual Reproduction

The vegetative stage of the Ascomycete life cycle is accompanied or followed by asexual sporulation by the production of single spores called conidia from the tips of aerial hyphae called *conidiophores* (Fig. 3). There are two possibilities in the mode of conidiospore formation.

3. The spores can be delimited by a complete transverse wall formation followed by spore differentiation (Fig. 3a) termed *thallic spore formation*.
4. More usually, the spores are formed by the extrusion of the wall from the hyphal tip, termed *blastic spore formation* (Fig. 3b).

These conidiospores are mostly single celled and contain one haploid nucleus, but in some cases they can be multinucleate and contain several haploid nuclei produced by mitosis.

Spores can be produced from single, unprotected conidiophores or they can be produced from aggregations that are large enough to be seen with the naked eye (Fig. 3c).

There are several modes of conidiophores aggregations.

- The conidiophores can aggregate into stalked structures where the spores produced are exposed at the top (*synnema* or *coremia*).
- In some cases, varying amounts of sterile fungal tissue can protect the conidia, as in the flaskshaped *pycnidia*.
- Some species produce conidia in plant tissue, and the conidial *acervulus*.

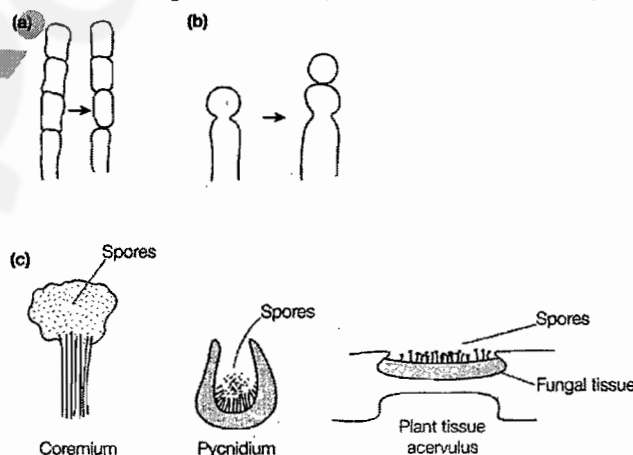


Figure 3: Asexual sporulation in Ascomycota

Sexual Reproduction

Sexual reproduction in Ascomycota occurs after fusion of different mating-type mycelia by any one of the following three modes.

- Mating cell fusion (in Yeasts)
- Gametangial contact
- Spermatisation
- Somatogamy

After sexual fusion generally a *dikaryotic phase* exists for a considerable period of time in most of the advanced member of this phylum. The fusion between the two haploid nuclei forms the diploid zygotic nucleus.

A transient diploid phase is rapidly followed by the formation of ascospores within sac-shaped asci differentiated from modified hyphal tips. In the initial stages of ascus development hooked hyphal tips form, called *croziers* or *shepherds' crooks* because of their shape. They have distinctive septae at their base which insures that two different mating-type nuclei are maintained in the terminal cell. Formation of the septae is coordinated with nuclear division (Fig. 4). In yeasts all these events occur within one cell, after fusion of two mating-type cells, the whole cell being converted into an ascus.

In more complex Ascomycetes many asci form together, creating a fertile tissue called a *hymenium*. In some groups the hymenium can be supported or even enclosed by large amounts of vegetative mycelium. The whole structure is called a fruit body or *sporocarp* and is used as a major taxonomic feature (Fig. 5). They can become large enough to be seen with the naked eye. Flaskshaped sexual reproductive bodies are called *perithecia*, cup-shaped bodies are called *apothecia* and closed bodies are called *cleistothecia*. These structures have evolved to protect the asci and assist in spore dispersal, but the hymenium itself is unaffected by the presence of water.

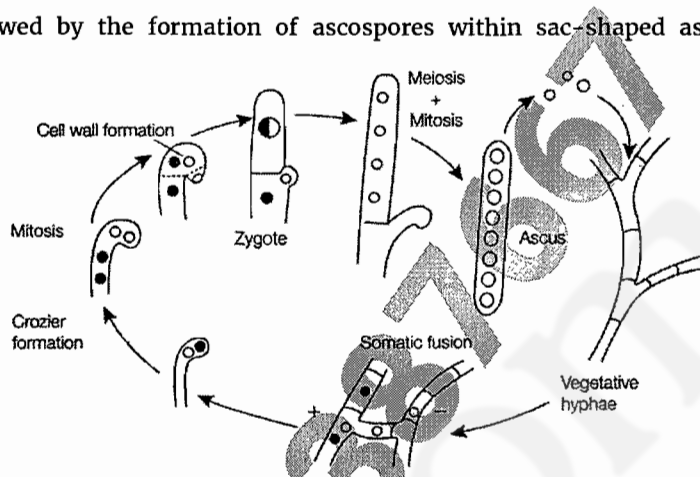


Figure 4: Sexual process in Ascomycetous Fungi

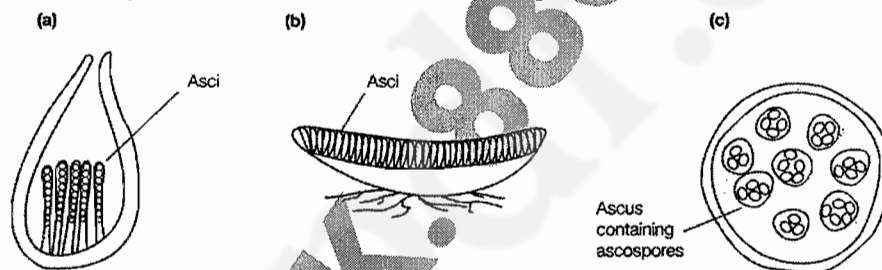


Figure 5: Three major types of sporocarps in Ascomycota. (a) Perithecium (b) Apothecium (c) Cleistothecium

Reproduction in Basidiomycota

This group of fungi are characterized by the most complex and large structures found in the fungi. They are also distinctive in that they *very rarely produce asexual spores*.

Much of the life cycle is spent as vegetative mycelium, exploiting complex substrates. A preliminary requisite for the onset of sexual reproduction is the acquisition of two mating types of nuclei by the fusion of compatible hyphae. The nuclei from two mating-type are held within every hypha compartment for extended periods of time. This is termed a *dikaryotic state*, and its maintenance requires elaborate septum formation during growth and nuclear division.

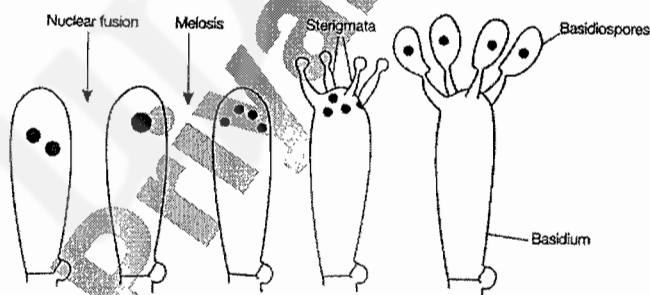


Figure 6: Basidium Formation

water. The hymenium is distributed over sterile, dikaryotic supporting tissues which protect it from rain.

Onset of sexual-spore formation is triggered by environmental conditions and begins with the formation of a fruit body primordium. Dikaryotic mycelium expands and differentiates to form the large fruit bodies or *sporocarps*, which we recognize as mushrooms and toadstools. Diploid formation and meiosis occur within a modified hyphal tip called a *basidium* (Fig. 6).

Four spores are budded from the basidium.

Basidia form together to create a hymenium which is highly sensitive to the presence of free

Chytridiomycota: A general account

An introduction to Chytridiomycota

Members of the phylum Chytridiomycota have unicellular to mycelial thalli. Their cell wall composition is chitinous, and true cellulose is not known to occur. As chitin cell wall composition is a conservative characteristic, this division was classified by Bartnicki-Garcia (1970) with the true fungi. Recent studies sequencing of the small subunit rRNA also support the affinity of this division with the true fungi. Thus, this division has been classified with the true fungi even though flagellated spores and gametes are produced. Other true fungi do not produce flagellated spores and gametes.

Gametes and zoospores have a single, posterior whiplash flagellum.

Sexual reproduction is variable and may be isogamous, anisogamous or oogamous. The division has a single class, the **Chytridiomycetes**.

Salient features

Noteworthy features of these fungi include the following.

1. They are the most primitive fungi.
2. Many chytrids are aquatic, mostly found in fresh water.
3. The thalli are coenocytic because the hyphae are non-septated.
4. All the nuclei in the somatic stage are haploid.
5. Some species are unicellular, e.g. *Synchytrium* sp.
6. The cell wall is made of true chitin.
7. They usually do not form the true mycelial structure. Some members have rhizoids in place of mycelium.
8. The mode of nutrition is mostly saprobic. However, certain species are parasitic and cause diseases in plants and animals.
9. These fungi produce motile cells as zoospores and gametes. They are the only "true" fungal phylum to produce flagellated cells.
10. The flagellated cells bear a single, whiplash flagellum that is inserted posteriorly.
11. Asexual reproduction is based mainly on zoospores.
12. During the sexual stage in most species, flagellated gametes are released and they unite together to form a Zygote.

Some well studied genera of this group include *Allomyces*, *Synchytrium*, *Blatocladia*, *Batrachomyces* etc.

Important Orders

Order: Chytridiales

Fungi in this order are commonly referred to as "chytrids". The thallus is commonly unicellular or may have limited hyphal growth, but is not considered to be mycelial. Hyphal cells are coenocytic except where there are reproductive structures.

The Chytridiales are thought to be the most primitive members of the Chytridiomycota. A distinctive characteristic of this order can be observed in the ultrastructure of the zoospore. Ribosome is loosely aggregated around the nucleus that is not enclosed in a nuclear cap.

Order: Blastocladales

Fungi in this order are more complex than the previous order.

True mycelium is produced, which is coenocytic. Zoospores in this order differ from those in the Chytridiales in that the ribosome that is around the nucleus is enclosed around a nuclear cap, which is an extension of the nuclear membrane.

Life cycles of the Chytridiomycota

Most chytrids have haploid zoospores and thalli but some Blastocladales show an alternation of haploid (gametothallic) and diploid (sporothallic) generations (Figure 1 shows the life cycle of *Allomyces*.)

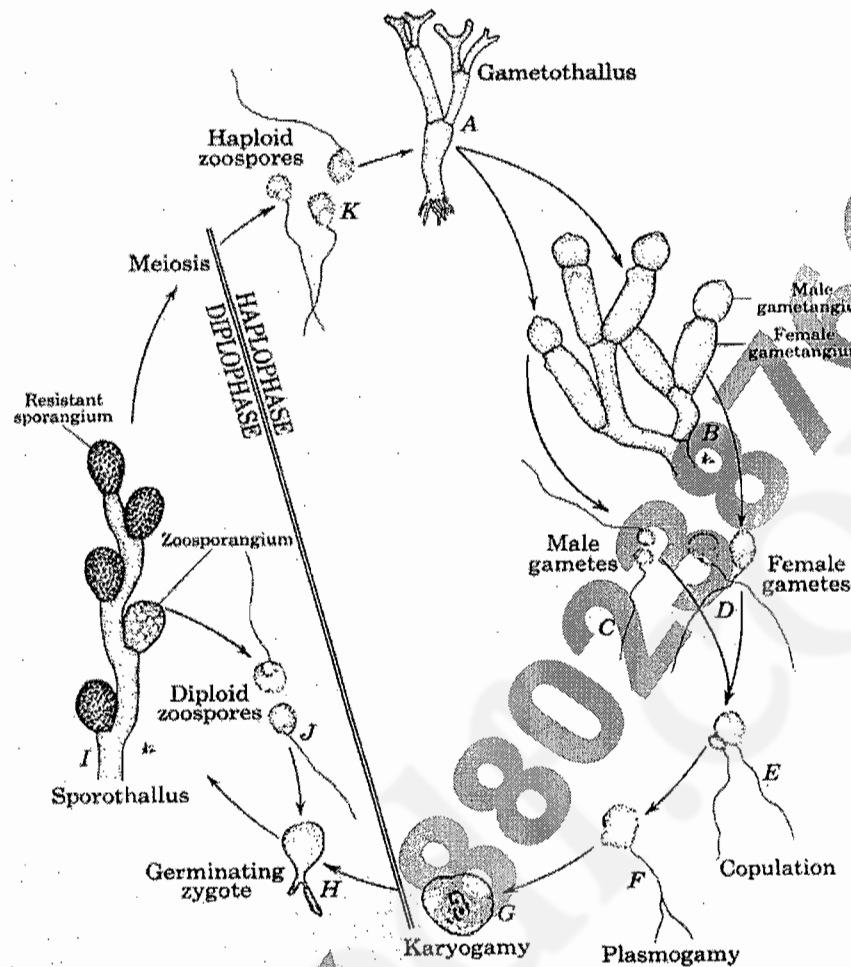


Figure 1: Life cycle of *Allomyces*

Sexual reproduction may occur in several different ways.

- In some chytrids it is by **gametogamy**, the fusion of gametes which are posteriorly uniflagellate.
- **Isogamous** conjugation occurs if there is no morphological distinction between the two fusing partners.
- In some Blastocladales (e.g. *Allomyces*) **anisogamy** takes place by fusion between a smaller, more actively motile male gamete with a larger, sluggish female gamete.
- **Oogamy**, fusion between an actively motile male gamete and a much larger, non-flagellate, immobile globose egg, is characteristic of Monoblepharidales.
- **Somatogamy**, the fusion of undifferentiated hyphae or rhizoids, has been well documented in cultures of the fresh-water fungus *Chytrium hyalinus*.
- Fusion of gametangia (**gametangiogamy**) has been reported for *Zygorhizidium planktonicum*, a parasite of the diatom *Synedra*.

Zygomycota

Introduction to Zygomycota

The Zygomycota contains approximately 1% of the described species of true Fungi (~900 described species; Kirk et al. 2001). These fungi are common in terrestrial and aquatic ecosystems.

Zygomycota are defined and distinguished from all other fungi by sexual reproduction via zygospores following gametangial fusion and asexual reproduction by uni-to-multispored sporangia within which nonmotile, single-celled sporangiospores are produced. The phylum comprises at least seven phylogenetically diverse orders.

Zygomycota are among the most ecologically diverse group of fungi, functioning as saprophytes on substrates such as fruit, soil, and dung (Mucorales), as harmless inhabitants of arthropod guts (Harpellales), as plant mutualists forming ectomycorrhizae (Endogonales), and as pathogens of animals, plants, amoebae, and especially other fungi (all Dimargaritales and some Zoopagales are mycoparasites). A number of species are used in Asian food fermentations, such as *Rhizopus oligosporus* in the Indonesian staple tempeh, and *Actinomucor elegans* in Chinese cheese or sufu.

Salient features of Zygomycota

1. Zygomycota, like all true fungi, produce cell walls containing chitin.
2. They grow primarily as mycelia, or filaments of long cells called hyphae.
3. Unlike the so-called 'higher fungi' comprising the Ascomycota and Basidiomycota which produce regularly septate mycelia, most Zygomycota form hyphae which are generally coenocytic because they lack cross walls or septa. There are, however, several exceptions and septa may form at irregular intervals throughout the older parts of the mycelium or are regularly spaced in two sister orders of Zygomycota, the Kickxellales and Harpellales.
4. The unique character of the Zygomycota is the zygospore. Zygospores are formed within a zygosporangium after the fusion of specialized hyphae called gametangia during the sexual cycle. A single zygospore is formed per zygosporangium. The mature zygospore is often thick-walled, and undergoes an obligatory dormant period before germination.
5. Most Zygomycota have a zygotic or haplontic life cycle. Thus, the only diploid phase takes place within the zygospore. Nuclei within the zygospore undergo meiosis during germination.
6. Zygomycota typically undergo prolific asexual reproduction through the formation of sporangia and sporangiospores. Sporangiospores are distinguished from other types of asexual spores, such as conidia of the Ascomycota and Basidiomycota, by their development. Walled sporangiospores are formed by the internal cleavage of the sporangial cytoplasm. At maturity, the sporangial wall typically disintegrates or dehisces, thereby freeing the spores that are usually dispersed by wind or water.
7. Sporangia are formed at the ends of specialized hyphae called sporangiophores. Two variant types of sporangia include sporangiola and merosporangia. **Sporangiola** are simply uni-to-few spored sporangia containing between 1-to-30 spores. **Merosporangia** are elongated sporangiola with uniseriate spores usually produced from a vesicle or stalk.
8. A unique sporangium type is the **trichospore**, a one-spored sporangium, produced by members of the Harpellales, which are endo-commensals living within the gut of arthropods, including terrestrial beetles and millipedes, fiddler crabs, and the larvae of many aquatic insects. Trichospores possess one to several basal hair-like filaments that likely aid in the attachment of the spores to debris and plants in aquatic ecosystems before they reenter the arthropod gut.
9. Like other Fungi, Zygomycota are heterotrophic and typically grow inside their food, dissolving the substrate with extracellular enzymes, and taking up nutrients by absorption. The most common members of the Zygomycota are the fast growing members of the Mucorales. They function as decomposers in soil and dung, thereby playing a significant role in the carbon cycle.
10. Zygomycota also participate in a number of interesting symbioses. As mentioned above, the Harpellales inhabit arthropods (particularly freshwater aquatic insect larvae) where they are attached to the chitinous lining of the hindgut. Harpellids feed on nutrients that are not utilized by the arthropod. Because they are generally assumed to neither harm nor benefit the host animal, this association is considered commensalistic.

11. Certain species of Zoopagales parasitize non-fungal hosts, such as nematodes, rotifers, and amoebae. The Endogonales are a unique group in the Zygomycota because some members can form ectomycorrhizal associations with pine roots, while others appear to be saprobic.

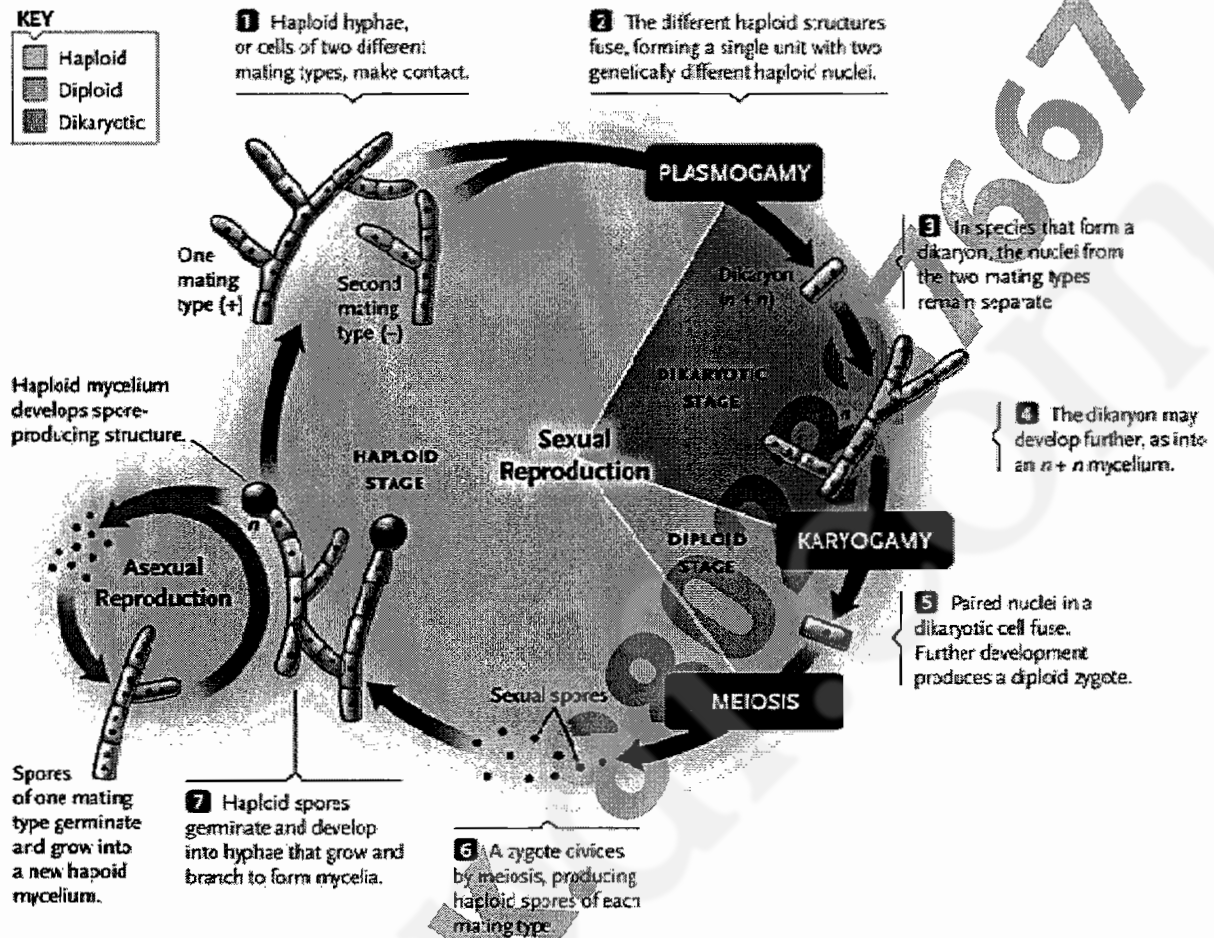


Figure 1: Generalized life cycle of Zygomycota. Asexual reproduction occurs primarily by sporangiospores produced by mitosis and cell division. The only diploid ($2N$) phase in the life cycle is the zygospore, produced through the conjugation of compatible gametangia during the sexual cycle.

Life cycle of Rhizopus

Introduction to the genus

Rhizopus is a genus of order Mucorales in the Phylum Zygomycota within kingdom Fungi. (Formerly treated under Zygomycetes within Zygomycotina by GC Ainsworth's system and other classical systems of fungal systematics).

Based on the modern systematics of Fungi (Patterson and Sogin 1992; Mims, Alexopoulos and Blackwell, 1998) the accepted systematic position of *Rhizopus* is as under:

- Kingdom: Fungi
- Phylum: Zygomycota
- Class: Zygomycetes
- Order: Mucorales
- Family: Mucoraceae
- Genus: *Rhizopus*

Rhizopus is also commonly called bread mold.

Rhizopus is a cosmopolitan filamentous fungus found in soil, decaying fruit and vegetables, animal feces, and old bread. While *Rhizopus* spp. are common contaminants of food, they are also occasional causes of serious (and often fatal) infections in humans and animals. Some species are plant pathogens.

Rhizopus spp. are among the fungi causing the group of infections referred to as zygomycosis. Although the term mucormycosis has often been used for this syndrome, zygomycosis is now the preferred term for this angio-invasive disease. *Rhizopus arrhizus* is the most common cause of zygomycosis.

Structure

- The common **black bread mold** (*Rhizopus stolonifer*) is a well studied species.
- The thallus shows mycelial organization. The basic structural unit is a hypha, that grows apically. The hyphae have true chitinous walls. They are aseptate.
- The mycelium is characterized by the presence of stolons and pigmented rhizoids, the formation of sporangiophores singly or in groups from nodes directly above the rhizoids, and apophysate, columellate, multi-spored, generally globose sporangia. After spore release the apophyses and columella often collapse to form an umbrella-like structure.
- Colonies are fast growing and cover an agar surface with a dense cottony growth that is at first white becoming grey or yellowish brown with sporulation.
- It produces three types of hyphae.
 1. *Stolon hyphae* spread over the surface of bread as the mycelium grows,
 2. *Rhizoid hyphae* penetrate the bread to digest it and to anchor the mycelium, and
 3. *Sporangiophores* are upright hyphae that form a sporangium at their tips.

Reproductive Cycle

Reproduction occurs both by asexual and sexual means.

Asexual reproduction

It takes place by the following methods:

1. **Fragmentation.** Vegetative hyphae break up into smaller fragments and each fragment develops into new mycelium.
2. **Chlamydospores.** These are thick-walled mycelial segments arising from the mycelium. These segments become round and secrete a thick wall. Chlamydospores can survive unfavourable conditions. Under suitable conditions they germinate to form new mycelium.
3. **Oidia.** These structures are formed by *Rhizopus oryzae*. They arise as terminal buds of the somatic hyphae and later get detached to generate new thalli.
4. **Spores.** (aplanospores or sporangiospores). This is the most common method of asexual reproduction. The sporangia develop terminally on hyphal branches, known as **sporangiophores** (Fig. 2) which may be simple or branched. The apex of aerial hypha swells and cytoplasmic mass along with nuclei move in this part. The swollen part enlarges and develops into large globose structure. This is the young sporangium. On maturity, contents of sporangium become differentiated into a thick dense layer of cytoplasm with many nuclei towards the peripheral region beneath the sporangial wall, and a vacuolated portion towards the centre. A dome-shaped septum is then laid down cutting off a distal, peripheral position (which will contain the spores) from a central cylindrical or subglobose spore-free core, the **columella**.

The contents of the distal portion become cleaved into 2- to 10- nucleate spores. When the sporangia are mature, the sporangiophores may be seen as coarser, blunt-tipped, aerial hyphae growing away from the substratum. The mature sporangium and sporangiophore becomes a stalked spore drop. The sporangial wall dissolves except for basal region where its remnants can be seen as a frill or collar at the base of columella. The spores are dark, usually elliptic to ovoid. When they fall on suitable substratum, in presence of proper moisture and temperature, they germinate by germ tubes to form new mycelium.

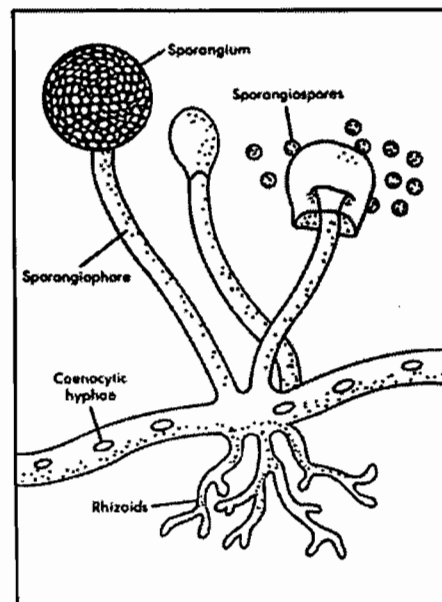


Figure 2: Sporulation in *Rhizopus*

Sexual reproduction

Sexual reproduction is isogamous, by the conjugation of two identical coengametangia (Fig. 3), to give rise to the zygospores. Some species are homothallic, but **most species are heterothallic**. In heterothallic species, zygospores are formed only when mycelia of compatible strains (+ and -) contact each other.

The two strains are morphologically identical. The phenomenon of **heterothallism** (i.e. occurrence of different compatible strains of mycelia) was discovered in Mucorales by **A.F. Blakeslee** in 1904. Because it was not possible to designate one strain as male and the other as female, Blakeslee labelled one strain as (+) and the other as (-). The two compatible strains are said to differ in **mating type**.

Zygophores of opposite mating types grow towards each other through the air, a form of positive **zygotropism**. When the opposite zygophores (+ and -) come in contact the walls fuse to each other firmly and zygophore elongation ceases. The two zygophores then swell in the region immediately adjacent to the area of contact to give two multinucleate **progametangia**. Dense cytoplasm and numerous nuclei flow to the contacting tips which enlarge further. A septum then separates the terminal **part-gametangium** from the remaining part of the progametangium, the **suspensor**.

Since gametangia are undifferentiated mass of multinucleate protoplast, they are called **coengametangia**. As the gametangia mature, the separating wall dissolves and the protoplasts of both mix with each other. Nuclear fusion occurs between nuclei of (+) and (-) strains to give numerous diploid nuclei in each **zygospore**.

During last recent years details of physiology of sexual reproduction in heterothallic Mucorales have become available. When two compatible strains approach each other, **three reactions** can be distinguished.

1. **Zygophore induction.** It is a **telemorphic reaction** which involves the induction of aerial zygophore formation. Zygophores in both (+) and (-) strains are induced by the hormones **trisporic acids B, C**. These acids are oxidised, unsaturated derivatives of trimethyl cyclohexane.
2. **Zygotropic reaction.** It involves directed growth of zygophores of (+) and (-) mating partners towards each other. It is a chemotropic response. Mesland *et al* (1974) have shown that the vegetative mycelium could produce volatile substances which induced zygophore development and the zygotropic reaction. These volatile substances have a dual role. They enhance trisporic acid synthesis in the opposite mating type, and also mediate the zygotropic response.
3. **Thigmotropic reaction.** It involves the events which occur after contact of the respective zygophores, such as gametangial fusion and septation.

The Zygospore: The young zygospore, containing many diploid nuclei lies within the parent gametangial wall. It later enlarges and secretes several layered thick wall around it. As the zygospore matures, it breaks up the original gametangial wall into fragments which fall apart exposing the outer, thick, spiny, dark exospore. The wall of mature zygospore is probably five-layered, two layers in the outer, **exospore** and three in the inner, **endospore**. The zygospore germinates after a long period of rest. During germination the outer wall cracks and the inner comes out in the form of a **germsporangiophore** which bears a single terminal **germsporangium**, containing many spores.

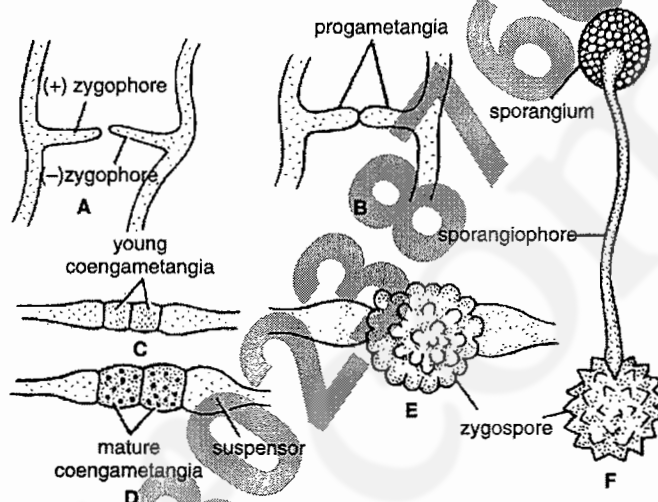


Figure 3: Sexual process in *Rhizopus*

Ascomycota – I: General account

Introduction to Ascomycota

The term sac fungi applies to the members of the Phylum Ascomycota of Kingdom Mycota (Alexopoulos *et al.*; 1998). They constitute the largest group of Fungi, including about 75% of the described fungal species. All members produce haploid spores after sexual reproduction within sac like closed structures, known as Ascus. These spores are called Ascospores, and they are the defining characteristic of Ascomycota.

The Phylum Ascomycota comprises of the following classes.

1. Hemiascomycetes
2. Plectomycetes
3. Loculoascomycetes
4. Pyrenomycetes
5. Laboulbeniomycetes
6. Discomycetes

In older classification, these fungi were grouped at the level of a subdivision Ascomycotina under the Division Eumycota by G.C. Ainsworth (*Fungi: An Advanced Treatise* in 1973).

Salient features

The important features of the Ascomycota are as follows.

1. The distinctive feature is formation of *Ascospores*. They are post sexual spores produced by meiosis inside sac like hyphal tips (*Ascus*)
2. Almost all members are multicellular and have hyphal organization. *Saccharomyces*, *Scizosaccharomyces* are however unicellular in the trophic stage.
3. The hypha is always septated but the septum is perforated.
4. The cell wall is made of true chitin and some lipid substances.
5. Unique cell structures:
 - a. *Woronin bodies*: A peroxisome-derived, dense core microbody with a unit membrane found near the septae that divide hyphal compartments in filamentous Ascomycota. One established function of Woronin-bodies is the plugging of the septal pores after hyphal wounding, which restricts the loss of cytoplasm to the sites of injury.
 - b. *Concentric bodies*:
 - i. Generally found in lichen forming Ascomycota
 - ii. Function unclear, may also be found in some non-lichen Ascomycota members (*Venturia inaequalis*)
 - iii. Present in the cell, when the fungus is in association with the algal partner
 - iv. Made of two parts:
 1. A translucent core, delimited by a membrane like structure
 2. A dense periphery with radiating filamentous structures
6. The growth pattern is almost always mycelial, sometimes showing complex hyphal aggregations such as *Sphacelia* and *Sclerotia* stages in *Claviceps*.
7. The nutrition is always absorptive.
8. The reserve food material is Glycogen.
9. The somatic phase is always haploid.
10. Asexual reproduction occurs by exogenously produced aplanospores, known as *Conidia* or *Conidiospores*. There is never a formation of zoospores or sporangiospores for asexual purposes.
11. Budding and fission is present as modes of asexual multiplication in the members of yeasts.
12. The sexual reproduction is divided into two parts, namely plasmogamy and karyogamy. Often there is a considerable lag between plasmogamy and karyogamy. Therefore, a *Dikaryon* stage is quite frequently found in Ascomycota.
13. Plasmogamy can be brought about by several methods, like Gametangial Contact, Gametangial Fusion, Spermatisation and Somatogamy. No gamete is ever motile in Ascomycota.

14. For plasmogamy, there is a self-incompatibility system in some species, like *Saccharomyces cerevisiae*, *Neurospora crassa* etc. This incompatibility system is called Heterothallism. It is considered to be an advanced sexual feature.
15. Some members like *Aspergillus* display deviation from the sexual mechanisms and undertake parasexual cycle.
16. In sexual reproduction, meiosis takes place immediately after karyogamy. Meiosis results into Ascospore formation within Ascus. The shared character that defines the Ascomycota is the ascus. Ascus is a hyphal segment that develops late during the sexual reproduction process. In this structure, first the parent nuclei fuse to give rise to the zygotic nucleus and later the zygotic nucleus undergoes meiotic division to produce ascospores. The ascospores are normally produced 8 in number because one round of meiotic division is followed by a single round of mitotic division. The ascospores are the mode of dispersal after the sexual reproduction in all the members of Ascomycota.
17. Asci are mostly associated with fruiting bodies known as Ascocarps. Important types of ascocarps are Cleistothecium, Perithecium, Loculothecium and Apothecium. The distribution of Ascocarps is a taxonomically important for classifying Ascomycota at the level of classes.
18. In some members sexual system is poorly developed. This is called *Degeneration of Sex*. Some non-sexually reproducing genera show *Parasexual Reproduction*. In sexually reproducing species, *heterothallism* may be seen. This is a physiological system which ensures that sexual reproduction takes place only in between genetically different thalli. This is an advanced feature as it increases genetic variability within the population.

The classes of Ascomycota

The members of Ascomycota are separated into 6 classes.

Class 1. Hemiascomycetes. Reduced ascomycetes, no ascocarp and ascogenous hyphae, asci naked, thallus reduced mycelial or yeast like, ascus development directly from the zygotic cell as ascogenous hyphae are not formed e.g., *Saccharomyces*, *Scizosaccharomyces*, and *Taphrina*. The members of this group are further unique in not having a continuous ascus vesicle, as found in the members of other classes of Ascomycota.

Class 2. Loculoascomycetes. Ascocarp and ascogenous hyphae present, the only class where the asci are two walled (bitunicated), thallus mycelial, fruiting body is **ascostroma** (a mass of tissue with locules) within which the locules containing asci are found e.g., *Venturia*, *Myriangium*, *Mycosphaerell*, *Pleospora*.

Class 3. Plectomycetes. Thallus mycelial, ascocarp and ascogenous hyphae present, ascus development from an ascogenous hypha, asci unitunicated (one walled) and scattered in fruiting body without any clear hymenial layer, fruiting body (ascocarp) is completely closed and called **Cleistothecium** e.g., *Penicillium*, *Aspergillus*.

Class 4. Pyrenomycetes – Ascocarp and ascogenous hyphae present, thallus mycelial, asci unitunicated and arranged in definite hymenium in ascocarp which is **perithecium** with ostiole, asci inoperculate with apical pore or slit; e.g., *Neurospora*, *Claviceps*, *Chaetomium*, *Xylaria*. In some members of Pyrenomycetes, a cleistothecium may be formed but such a cleistothecium has a well defined hymenium and the asci are regularly arranged with the hymenial layer rather than being scattered as seen in the cleistothecium of Plectomycetes.

Class 5. Laboulbeniomyces. Ascocarp and ascogenous hyphae present, thallus reduced, asci one walled and arranged regularly in ascocarp which is of cellular **perithecium** type, asci inoperculate, mainly exoparasites on arthropods e.g., *Laboulbenia*, *Herpomyces*, *Rhizomyces*, *Ceratomyces*, *Stigmatomyces*. The perithecium in this class has highly characteristic structure, with three basal cells, two layered wall composed of 4 to 6 horizontal rows of cells and 4-5 vertical rows of cells. The asci arise in tufts, which are called Fascicles

Class 6. Discomycetes. Macroscopic fungi, ascocarp and ascogenous hyphae present, asci arranged regularly in a cup or saucer-shaped ascocarp called **apothecium**, asci unitunicated, operculate with apical pore, thallus mycelial, e.g., Cup fungi like *Peziza*, Morels (*Morchella*), Truffles like *Tuber*. Generally the ascocarp in this group is highly differentiated into multiple layers.

Ascocarps

Introduction to ascocarps and their basic types

An ascocarp, or ascoma, is the fruiting body (sporocarp) of an ascomycete fungus. It consists of very tightly interwoven hyphae and contain a large number of asci. The asci are sac like closed structures, each of which contains typically eight ascospores in most species.

The ascocarp is a supportive and protective structure for the asci in ascomycetous fungi. Their formation begins soon after plasmogamy and they assume the mature structure by the time the spores are ready to be released.

There are four morphological types of ascocarps, described below (Fig. 1).

1. **Cleistothecium:** In this case the ascocarp is round and closed with the hymenium enclosed, so the spores do not automatically get released. Cleistothecia are found in *Penicillium*, *Aspergillus* etc.
2. **Loculothecium**, also called **Pseudothecium:** This is an enclosed structure with several locule containing asci. The asci are not regularly organised into a hymenium and they are bitunicate, having a double wall which expands when it takes up water and shoots the enclosed spores out suddenly to disperse them. It is found in genera like *Venturia*, *Myriangium*, *Mycosphaerella*, *Pleospora*.
3. **Perithecium:** This has the shape of a flask. Its distinguishing feature is that on top it has a small pore, the **ostiole**, through which the spores are released one by one when ripe. Perithecia are found for example on *Neurospora*, *Claviceps*, *Xylaria* and *Nectria*.
4. **Apothecium:** Here the ascocarp is open like a cup. The fertile layer is free, so that many spores can be dispersed simultaneously. They are found in genera like *Peziza*, *Morchella*, *Helvella* and *Gyromitra*.

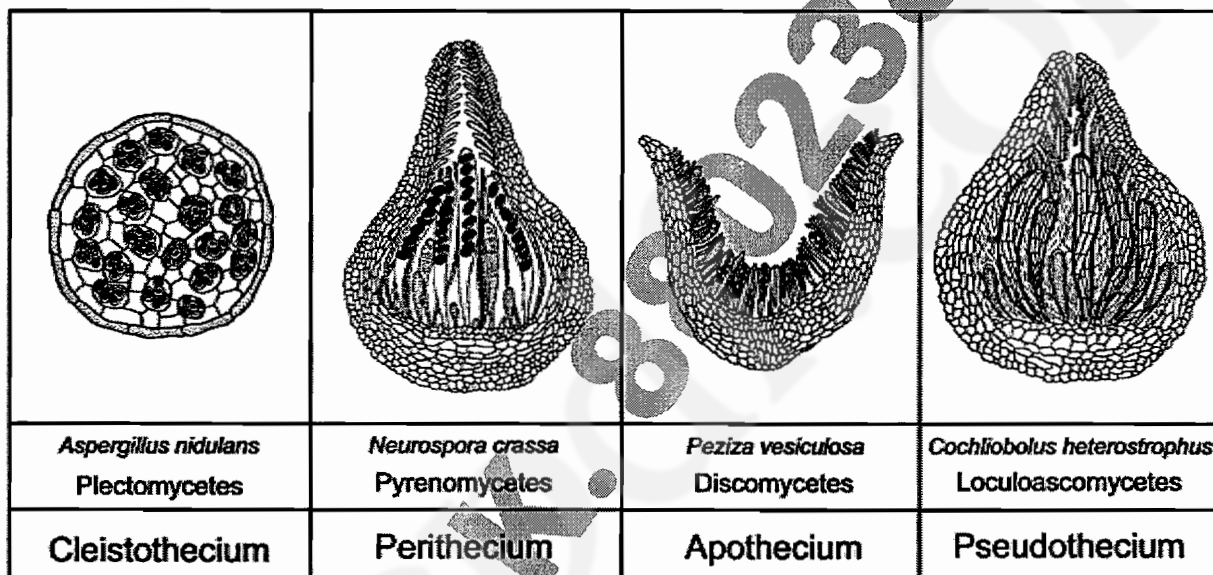


Figure 1: Fruiting bodies of filamentous ascomycetes

Six classes of Ascomycota are recognised by mycologists. The main criterion behind classification of Ascomycota is the type of ascocarp found in each group, as described below.

Class 1. Hemiascomycetes. No ascocarp and ascogenous hyphae.

Class 2. Loculoascomycetes. Ascostroma (a mass of tissue with locules) called Loculothecium or Pseudothecium

Class 3. Plectomycetes. Cleistothecium

Class 4. Pyrenomycetes - Perithecium with ostiole

Class 5. Laboulbeniomyces. Cellular perithecium

Class 6. Discomycetes. Apothecium

Formation of ascospores

Sexual reproduction in the Ascomycota leads to the formation of the *ascus* and ascospores. These structures define this fungal group and distinguishes it from other fungal phyla. The ascus is a tube-shaped vessel, a *meiosporangium*, which contains the sexual spores produced by meiosis and which are called *ascospores*.

Apart from a few exceptions, such as *Candida albicans*, the ascomycetes are haploid, i.e., they contain one set of chromosomes per nuclei. During sexual reproduction there is a diploid phase which commonly is very short, and meiosis restores the haploid state.

The basic account of the ascomycetous sexual process is outlined below.

The sexual process in the members of ascomycota is divided into two parts.

1. **Plasmogamy:** This is the first part of the sexual process, during which the union of protoplasm occurs without the fusion of the nuclei. In the case of *homothallic* species, mating is enabled between hyphae of the same fungal clone, whereas in *heterothallic* species, the two hyphae must originate from fungal thalli that are of a different mating type.

There are four methods to achieve plasmogamy among the Ascomycota members. These are:

- a. **Gametangial Contact:** In this, the compatible gametangia fuse selectively within a small region and a tube like structure is established. Through this tube like structure, the male nucleus or nuclei pass into the female reproductive structure that is known as Ascogonium. After this transfer of the male reproductive material the contact tube is broken down and the two gametangia separate. It is seen in genera like *Penicillium*, *Aspergillus*, *Claviceps* etc.
 - b. **Gametangial fusion:** This method involved total fusion of the gametangia to mix the sexually compatible protoplasts. It is seen in *Saccharomyces* spp.
 - c. **Spermatisation:** In this method, a small detachable cell (known as Spermatium) containing one or more male nuclei fuses with the receptive part of the female reproductive structure, known as Trichogyne. This is seen in *Neurospora*.
 - d. **Somatogamy:** In this, two ordinary somatic hyphae fuse in specific regions to bring about plasmogamy. It is seen in genus like *Peziza*.
2. **Karyogamy:** In Ascomycota, plasmogamy is not immediately followed by karyogamy. The nuclei from the two hyphae form pairs, initiating the *dikaryophase* of the sexual cycle. During this time, the pairs of nuclei synchronously divide. From the fertilized ascogonium, *dinucleate* hyphae emerge in which each cell contains two nuclei. These hyphae are called *ascogenous* or fertile hyphae. They are supported by the vegetative mycelium containing *uninucleate* hyphae, which are sterile. The mycelium containing both sterile and fertile hyphae may grow into fruiting body, the *ascocarp*, which may contain millions of fertile hyphae.

Each fertile hypha bears at its tip a characteristic U-shaped hook known as **Crozier**. The two nuclei contained in the crozier part of each hypha divide in such a way that the threads of their mitotic spindles run parallel, creating two pairs of genetically different nuclei. One daughter nucleus migrates close to the hook, while the other daughter nucleus locates to the basal part of the hypha. The formation of two parallel cross-walls then divides the hypha into three sections:

1. one at the hook with one nucleus
2. one at the basal of the original hypha that contains one nucleus, and
3. one that separates the U-shaped part which contains the other two nuclei.

Karyogamy occurs in the last of the above described segments, which is dikaryotic. This cell is now called ascus. It is in the ascus, where a diploid nucleus forms.

The terminal cell of the crozier curves round and fuses with the stalk cell, and this region of the ascogenous hypha may grow on to form a new crozier in which the same sequence of events is repeated. Repeated proliferation of the tip of the crozier can result in a tight cluster of asci in many ascomycetes.

3. **Sporogenesis:** The diploid nucleus goes through meiosis, forming four haploid nuclei. In most species, these haploid nuclei go through additional round of division, that is by mitosis. This results into eight nuclei. Each nucleus gathers cytoplasm around it and also two membranes, one from the invagination of the plasma membrane and one derived from the endomembrane system of the ascus cell. The two membranes that initially surrounds the spore nucleus are collectively called *Prospore Membranes*.

Later, these membranes completely surround individual nuclei into separate cells. In the last stage, a chitinous wall is laid down between the two membranes. The inner membrane forms the plasma membrane of the ascospore and the outer membrane degenerates. Secondary wall material is secreted within the primary wall. Now, the spore is considered to be mature and ready for release.

The ascospores are aligned inside the ascus like peas in a pod. Upon opening of the ascus, ascospores may be dispersed by the wind, while in some cases the spores are forcibly ejected from the ascus; certain species have evolved spore cannons, which can eject ascospores up to 30 cm. away. When the spores reach a suitable substrate, they germinate, form new hyphae, which restarts the fungal life cycle.

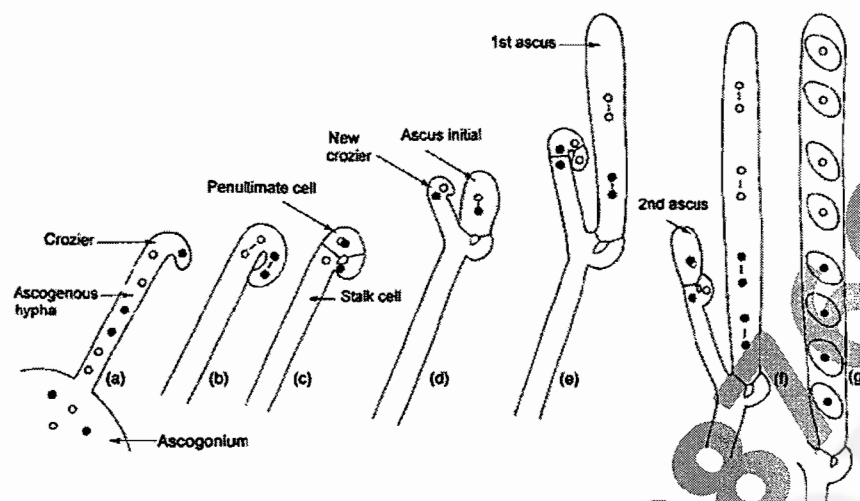


Figure 2: Diagrammatic representation of cytological features during ascus development. (a) Ascogonium hypha with a crozier at its tip developing from an ascogonium. (b) Conjugate nuclear division of the two nuclei in the crozier. (c) Two septa have cut off a binucleate penultimate cell. The two nuclei fuse to form a diploid nucleus. The uninucleate terminal segment of the ascogonium hypha has recurved and fused with the ascogonium hypha to form the stalk cell. (d) The penultimate cell enlarges to become an ascus initial within which the fusion nucleus begins to divide meiotically. A new crozier is developing from the stalk cell. (e) Second division of meiosis has occurred in the developing ascus. The behaviour of the new crozier repeats that of the first. (f) Mitotic division of the four haploid nuclei resulting from meiosis in the first ascus. (g) Ascospores formed.

Ascomycota – II: Life cycles

Saccharomyces

Importance of Saccharomyces

Saccharomyces, — a member of Hemiascomycetes — commonly known as **yeasts** due to unicellular structure, is represented by about 40 saprophytic species found ubiquitously, mostly on media rich in sugar or an organic matter of vegetable origin. The name *Saccharomyces* means 'sugar fungi'.

According to the Modern Fungal Systematics, the taxonomic position of the species is as follows:

Kingdom: Fungi
Phylum: Ascomycota
Subphylum: Saccharomycotina
Class: Saccharomycetes
Order: Saccharomycetales
Family: Saccharomycetaceae
Genus: *Saccharomyces*
Species: *S. cerevisiae*

The genus is of significant importance economically and also from research view point.

1. *Saccharomyces cerevisiae* and *Saccharomyces bayanus* are used in making wine and beer. Brewing yeasts are mostly polyploid. Yeasts is used to convert the sugars present in grape juice or must into alcohol by fermentation.
2. *Saccharomyces* can form symbiotic matrices with bacteria, and are used to produce kefir and ginger beer.
3. Yeast is used in nutritional supplements popular with health conscious, where it is often referred to as "nutritional yeast". It is a deactivated yeast, usually *Saccharomyces cerevisiae*. It is an excellent source of protein and vitamins, especially the B-complex vitamins, whose functions are related to metabolism as well as other minerals and cofactors required for growth. It is also naturally low in fat and sodium.
4. *Saccharomyces cerevisiae*, is also used widely in baking as a leavening agent, where it converts the fermentable sugars present in the dough into carbon dioxide. This causes the dough to expand or rise as the carbon dioxide forms pockets or bubbles. When the dough is baked it "sets" and the pockets remain, giving the baked product a soft and spongy texture.

5. *Saccharomyces boulardii*, used in medicine. More recently, *Saccharomyces boulardii* has been shown to be a sub-species of *Saccharomyces cerevisiae*.
6. The ability of yeast to convert sugar into ethanol has been harnessed by the biotechnology industry, which has various uses including ethanol biofuel. The process starts by milling a feedstock, such as sugar cane, sweetcorn, or cheap cereal grains, and then adding dilute sulfuric acid, or fungal alpha amylase enzymes, to break down the starches into complex sugars. A glucoamylase is then added to break the complex sugars down into simple sugars. After this, yeasts are added to convert the simple sugars to ethanol, which is then distilled off to obtain ethanol up to 96% in concentration.
7. *Saccharomyces* yeasts have been genetically engineered to ferment xylose, one of the major fermentable sugars present in cellulosic biomasses, such as agriculture residues, paper wastes, and wood chips. Such a development means that ethanol can be efficiently produced from more inexpensive feedstocks, making cellulosic ethanol fuel a more competitively priced alternative to gasoline fuels.
8. Apart from commercial uses, *Saccharomyces* is also the most intensively studied eukaryotic model organisms in molecular and cell biology, much like *Escherichia coli* as the model prokaryote. This is largely because the cell cycle in a yeast cell is very similar to the cell cycle in humans, and therefore the basic cellular mechanics of DNA replication, recombination, cell division and metabolism are comparable. It is particularly useful in studying the cell cycle because it is easy to culture.
9. Many proteins important in human biology were first discovered by studying their homologs in yeast; these proteins include cell cycle proteins, signaling proteins, and protein-processing enzymes.
10. *S. cerevisiae* was the first eukaryotic genome that was completely sequenced.

On 24 April 1996 *S. cerevisiae* was announced to be the first eukaryote to have its genome, consisting of 13 million base pairs, fully sequenced as part of the Genome project. The genome of *S. cerevisiae* is divided up into 16 chromosomes ranging in size between 250 kb and >2500 kb. The genome is composed of about 13,000,000 base pairs and 6,275 genes, although only about 5,800 of these are believed to be true functional genes. It is estimated that yeast shares about 23% of its genome with that of humans.

The yeast genome database is highly annotated and remains a very important tool for developing basic knowledge about the function and organization of eukaryotic cell genetics and physiology.

Asexual cycle of *Saccharomyces*.

Saccharomyces reproduces asexually by the following vegetative means.

1. By budding. It is the most common method of reproduction under favourable conditions. The protoplast of yeast cell bulges out in the form of a bud. The nucleus divides into two daughter nuclei and of these one migrates into the enlarging bud. The bud grows and is eventually separated from the mother cell. As the bud separates a scar with a convex surface is left on the parent cell. This scar is called bud scar. A concave scar is also retained by the newly formed daughter cell. It is called birth scar. This process of the formation of new buds from the parent cell continues and each time a bud scar is left. Sometimes, a large number of buds develop without being detached from one another and as the result chain of cells is formed. Such chains may be branched or unbranched and give the appearance of a false mycelium, called pseudomycelium. The cells of the pseudomycelium are loosely attached and they finally detach.

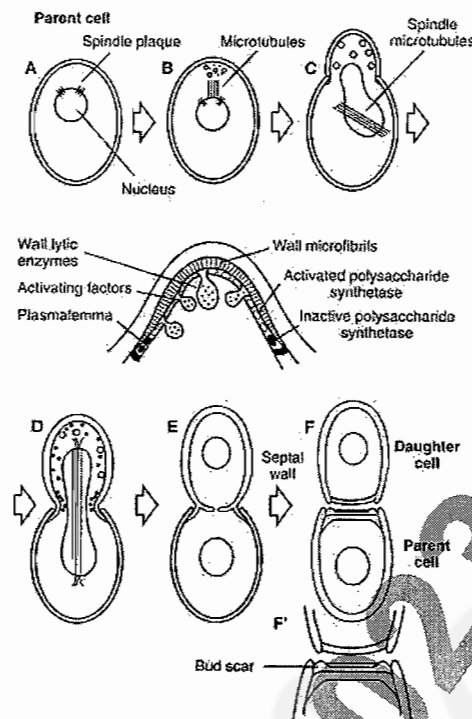


Figure 1: Budding in *Saccharomyces*

Molecular Mechanism of Budding

Budding is a highly regulated process. It has been widely studied to understand the regulation of eukaryotic cell cycle. The main regulatory molecule is an enzyme that transfers phosphoryl group to cell cycle related proteins and governs their timely activity. This enzyme is also called *Cyclin-dependent kinase* because it needs another protein called *Cyclin* to phosphorylate its target proteins.

There is only one cyclin-dependent kinase in *Saccharomyces*, encoded by the gene *Cdc28*.

It was discovered by Leland Hartwell in 1960s, who later received Nobel Prize for his studies in cell cycle regulation. This enzyme binds to different cyclins at different stages of cell cycle, shown below in Table 1.

In addition to cyclins and CDK, other proteins governing budding are:

1. The bud-site selection proteins (*BUD* proteins)
2. The proteins for bud formation (such as *CDC24*, *CK1*, *CDC42*, *BEM1*)
3. *Septins*: They form a ring of proteins, which are involved in positioning cell division in a proper way and cutting off the daughter bud precisely.

S. CEREVISIAE

CDK (one only)	Cdc28
Mid G ₁ cyclin	Cln3
Late G ₁ cyclins	Cln1, Cln2
Early S-phase cyclins	Clb5, Clb6
Late S-phase and early mitotic cyclins	Clb3, Clb4
Late mitotic cyclins	Clb1, Clb2

TABLE 1: Cell cycle regulatory molecules in *S. cerevisiae*

2. By **fission**. Fission is simple splitting of a cell, taking place only in the genus *Schizosaccharomyces*, into two daughter cells by the constriction and formation of a transverse wall. The parent cell elongates and its nucleus divides by intranuclear mitosis. Then a constriction appears somewhere near the middle of the mother cell, followed by a transverse septum, and as such two uninucleate daughter cells are formed.

3. By **endospore formation**. During unfavourable conditions, thick walled endospores are formed. The protoplast usually divides into four parts; each part is surrounded by a thick wall. These structures are

known as endospores. The endospore may survive adverse conditions and on the return of favourable conditions it germinates by budding and produces a chain of cells.

Sexual cycle of *Saccharomyces*

Sexual reproduction usually takes place during unfavorable conditions when the food supply is exhausted. Sex organs are entirely absent and as such the sexual reproduction is a simple process. In this process either two somatic cells or two ascospores are involved which assume the function of copulating gametangia. The zygote formed by their fusion eventually develops into an ascus which contains 4 or 8 ascospores depending upon the number of nuclear divisions.

Three types of life cycle patterns have been recognized in yeasts.

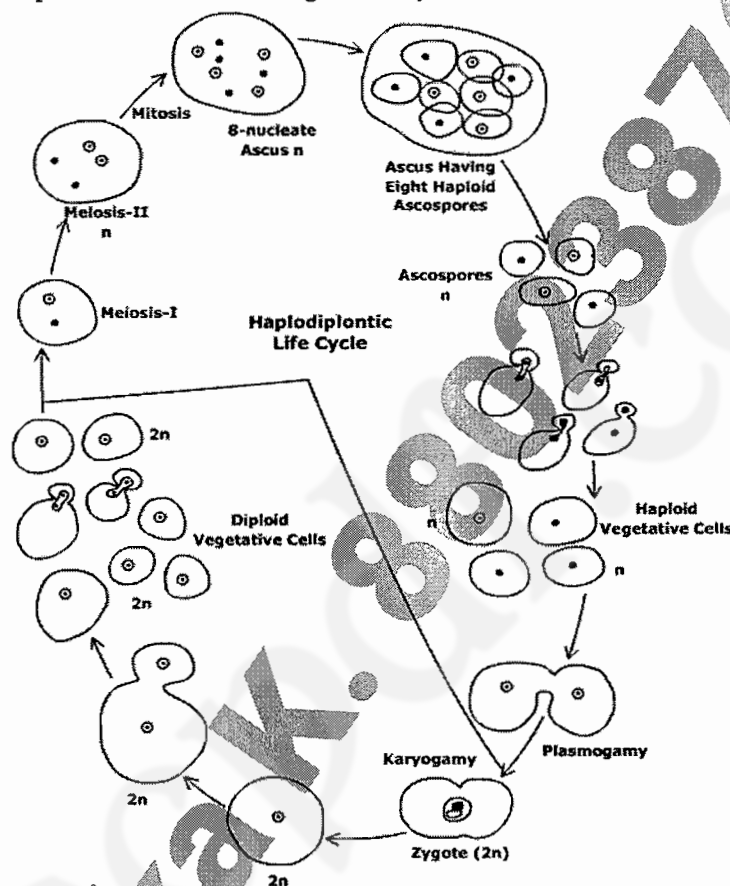
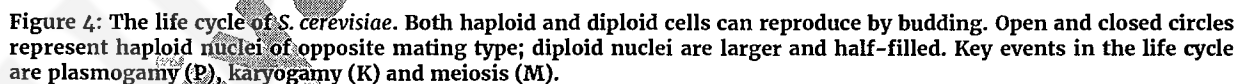


Figure 2: Haplontic life cycle in *Saccharomyces octosporus*

1. Haplobiontic type. This type of life cycle is found in *S. octosporus*. Here the haplophase is very elaborate and the diplophase is very short, confined to the zygote cell only. The somatic cells are haploid and function as a potential gametangia. At the time of sexual reproduction, two somatic cells meet in pairs, each sending a small protuberance towards the other. Both the protuberances come in contact and the wall at the point of contact dissolves to form a common passage, called conjugation tube. Thereafter, the nuclei of both the gametangia move into the conjugation tube; they fuse there and form a diploid zygotic nucleus. The zygote functions as an ascus. It undergoes meiosis immediately after karyogamy and thus eight haploid nuclei are formed. These nuclei organize themselves into ascospores. The ascospores are liberated by breaking of the ascus wall. The ascospore behaves like a somatic cell.

2. Diplobiontic type. This type of life cycle is represented by *S. ludwigii*. In this type, the diploid somatic stage is long and the haplophase, represented by ascospores, is very short. Only four ascospores are formed in an ascus, and they are not liberated from the ascus. The ascospores behave as gametangia and they copulate in pairs and each pair by fusion produces a diploid zygote cell within the ascus wall. The zygotic diploid cell produces a germ tube that pushes through the ascus wall. The germ tube becomes multicellular and functions as a diploid sprout mycelium. The new diploid yeast cells are budded from it. The diploid buds are detached from the parent sprout mycelium and function as diploid sprout cells. Under favourable conditions, these cells function as asci; each producing four ascospores as the result of a reduction division.



Introduction to the Genus

Penicillium, commonly known as blue or green mold, has over 100 species which are distributed all over the world. The genus derives its name from the Latin word *penicillus*, which means artist's brush, as the structure of its conidiophore resembles to that of a artist's brush.

The species of *Penicillium* are saprophytes and are found growing on rotting fruits, vegetables, meat, and other food stuff, and on textiles, leather, paper, etc. Most of the species are harmful as decaying agents, e.g.,

- *P. divaricatum* causes decay of wood.
- *P. chrysogenum*, *P. rubrum* and *F. purpurogenum* are responsible for the spoilage of paper and paper products.
- *P. citrinum* is one of the common causes of fungus 'fouling' of optical instruments.

However, a large number of *Penicillium* spp. also offer beneficial products.

- *P. digitatum*, *P. italicum* and *P. chrysogenum* are the source of the antibiotic Penicillin, the first 'wonder drug' of the world;
- The antibiotic griseofulvin (which is widely used as an antifungal agent) is obtained from *P. griseofulvum*.
- *P. roqueforti* and *P. camembertii* are used in the hydrolysis of fats and for flavouring of cheese.
- *P. chrysogenum* and *P. vitale* are used as a source of the enzyme glucose oxidase, which is used in ferment technology and food industry.

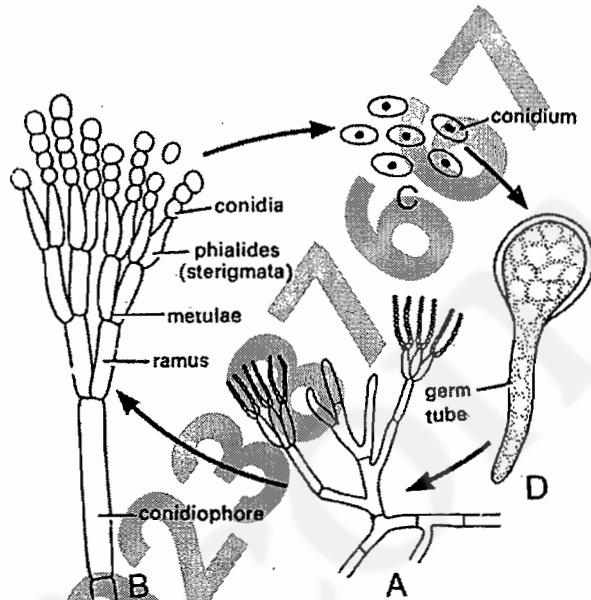


Figure 1: Somatic Structure and Conidiation in *Penicillium*

Somatic Structure

The mycelium is profusely branched, incompletely septate and is composed of thin walled and generally multinucleate hyphal cells. There is a central pore in each septum through which the cytoplasm of adjacent cells remains continuous. The mycelium mostly grows superficially, but some hyphae may penetrate deeper into the substratum to absorb food materials. The reserve food is in the form of oil globules.

The hyphae are hyaline and colourless in somatic phase but their walls develop pigments in the reproductive phase.

In some species of *Penicillium*, the mycelium becomes heterokaryotic because of networking between two mycelia. In others, compact, discrete, thick-walled pseudoparenchymatous structures, known as *sclerotia*, are also formed.

Asexual Reproduction

Asexual reproduction takes place by the formation of conidia (Fig. 1). The conidia are formed in long basipetal chains on specialized branches, called conidiophores.

The conidiophore arises as an erect branch from any cell of the mycelium. The conidiophores usually divide a few times and form the branches of different orders (primary, secondary, tertiary, etc.). The ultimate branches are known as metulae. A cluster of flask-shaped sterigmata (phialides) develops at the tip of each metulae. In some species of *Penicillium* (e.g., *P. expansum*) the metulae are also formed on the small branches of conidiophores which are known as rami (singular = ramus).

In *P. claviforme*, many conidiophores aggregate to form a compound club-shaped fructification, called *coremium*. The conidia formed on the coremium are known as *coremiospores*.

Development of conidia. The tip of the sterigma swells a little and at the same time, its nucleus divides to form two daughter nuclei. One of the daughter nuclei migrates into the swollen tip of the sterigma and then this portion separates from rest of the sterigma by the formation of a septum. In this way a uninucleate conidium is formed. The tip of the sterigma below the first formed conidium elongates, the nucleus divides and the second conidium is formed in the same way as the first. This process of conidium formation is repeated several times and a chain of conidia is formed. The youngest conidium is at the base of the chain and the oldest at the tip. This basipetal arrangement helps in the dissemination of conidia and provides proper nutrition to the developing conidia.

Mature conidia are green, blue or yellow in colour or hyaline. They are dispersed by wind and germinate on a suitable substratum. The nucleus of the conidium divides repeatedly and then a germ tube arises. All nuclei migrate into the germ tube. As the germ tube elongates, formation of septa takes place. In this way a new mycelium is formed.

Sexual Reproduction

Sexual reproduction has been studied only in some species of *Penicillium*. They show a gradual reduction in sexuality. Both the male and female sex organs are functional only in some of the sexually reproducing species.

However, in some (e.g., *P. vermicutatum*) although both the sex organs are present, the antheridium does not take part in sexual process.

The nuclei or the ascogonium only fuse in pair to form ascogenous hyphae. In others (e.g., *P. stipitatum*), sex organs do not develop at all and these species show the phenomenon of somatogamy.

The following account deals with the process of sexual reproduction in *P. vermicutatum* (= *Talaromyces vermicutatum*). (Fig. 2). The male and female sex organs are known as *antheridium* and *ascogonium* respectively. Both develop on the same hypha or on two adjacent hyphae of the same mycelium.

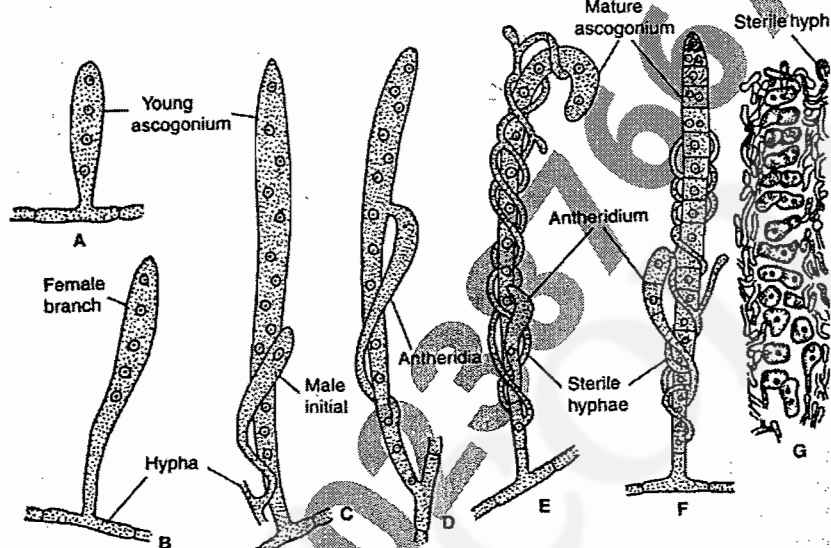


Figure 2: *Penicillium vermicutatum*. Showing stages in sexual reproduction and post-autogamy changes. A-D, stages in the development of sex organs; E, sexual apparatus surrounded by sterile hyphae arising from its base; F, establishment of dikaryons and septation of the ascogonium; G, later stage.

Ascogonium develops as an erect unicellular branch from any cell of the mycelium. The young ascogonium is uninucleate, but the nucleus undergoes repeated mitotic divisions as the ascogonium matures. Thus, a mature ascogonium has 32 or 64 daughter nuclei and it remains unicellular that is without any septation.

Following the initiation of ascogonium, an antheridial branch also develops from the same hypha on which ascogonium is initiated. As the antheridium grows, it coils spirally around the ascogonium. The tip of the antheridial branch touches the ascogonium and the intervening walls at the point of contact dissolve. The nucleus does not migrate into the ascogonium, probably it degenerates. Therefore, some authors have regarded the antheridial branch as Sterile Hypha. As a result, the sexual process can be regarded as Autogamy. However, there is mixing of cytoplasm of the antheridium and the ascogonium. This is followed by the segmentation of the ascogonium. The binucleate cells thus formed show uniseriate arrangement. This structure is comparable to *ascogenous hypha*, which is multicellular with dikaryotic cells. It is noteworthy that the dikaryotic cells have a single genetic type of nuclei.

The terminal cell of ascogenous hypha functions as *ascus mother cell*. The two nuclei of the dikaryon present in the ascus mother cell fuse to form a diploid nucleus.

The diploid nucleus first undergoes a meiotic division, followed by a mitotic division. This results in the formation of eight haploid nuclei. Each nucleus gets surrounded by cytoplasm and develops into an *ascospore*. Ascospores are mostly globose in shape and develop moderately thick walls around themselves.

Simultaneously with the development of ascogonium and ascogenous hyphae, some sterile hyphae develop around the ascogonium and they form a multilayered protective covering, the *peridium*. The ascogonium and ascogenous hyphae surrounded by the peridium form a fruiting body, known as *ascocarp*. The fruiting body is a completely closed structure of *cleistothecium* type. There are many asci that are irregularly placed within the ascocarp (a distinctive character of *Plectomycetes*).

At maturity, the ascus wall dissolves and ascospores float in the nutritive fluid present in the cleistothecium. Ascospores are liberated by the decay of the cleistothecial wall. They germinate on a suitable substratum and form new mycelia.

Aspergillus

Introduction

Aspergillus is a ascomycetous genus of filamentous fungi with about 200 species found worldwide. It is mostly saprobic and for many species, the natural habitat is in hay and compost.

The mycelium is well developed, profusely branched, and septate with a central perforation and consists of interwoven mass of hyphae with chitinous wall. The cells of the hyphae are multinucleate and mostly only one type of nuclei are present, i.e., the hyphae are homokaryotic. The species of *Aspergillus* mostly have some characteristic pigments in hyphae, conidiophores and conidia. The cytoplasm contains mitochondria, ER and ribosomes.

Aspergillus was first catalogued in 1729 by the Italian priest and biologist Pietro Antonio Micheli, who also named the genus due to the shape of the conidiophore that resembles an aspergillum (holy water sprinkler).

According to the traditional systematic plan as given by G.C. Ainsworth in 1972, the taxonomic status of the genus is as follows:

- Kingdom: Fungi
- Division: Eumycota
- Subdivision: Ascomycotina
- Class: Plectomycetes
- Family: Aspergillaceae (or Eurotiaceae)
- Genus: *Aspergillus*

The modern systematics of fungi, classifies the genus in the following way:

- Kingdom: Fungi
- Phylum: Ascomycota
- Class: Eurotiomycetes
- Order: Eurotiales
- Family: Trichocomaceae
- Genus: *Aspergillus*

Ecology

Aspergillus species are highly aerobic and are found in almost all oxygen-rich environments, where they commonly grow as molds on the surface of a carbon-rich (mainly carbohydrate and protein) substrates, as a result of the high oxygen tension. *Aspergillus* species are common contaminants of starchy foods, for example bread and potato, and grow in or on many plants and trees. Many species of *Aspergillus* demonstrate oligotrophy.

Some species of *Aspergillus* can also contaminate the lab cultures, due to which they are also referred to as the weed of the lab.

Reproduction

Aspergillus species reproduce using both asexual and sexual means. Asexual reproduction is favoured during the conditions of sufficient food reserves and optimal moisture.

Asexual reproduction

Aspergillus reproduces asexually by conidiation. (Fig. 1)

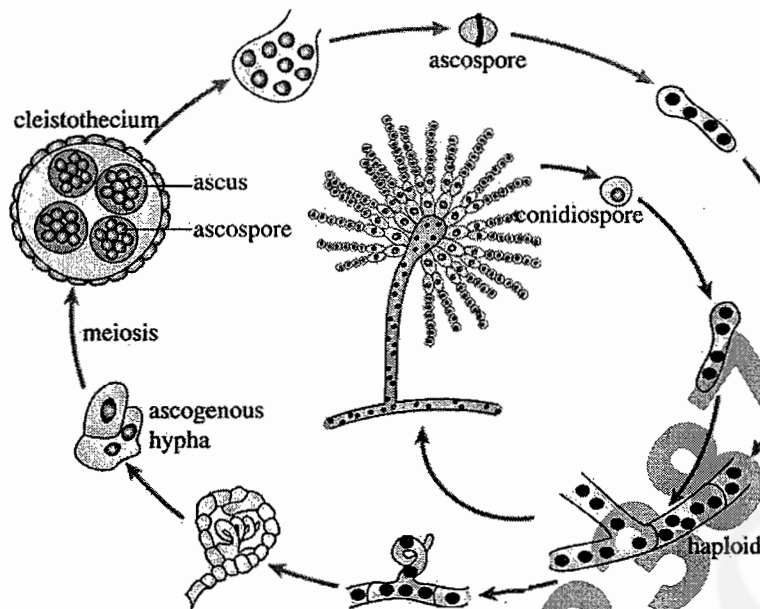


Figure 1: *Aspergillus* life cycle

Conidiospores develop very much like those in *Penicillium*. A conidiophore develops under rich nutrient conditions on a somatic hypha, once the latter has attained a certain degree of maturity.

The conidiophore develops on a foot cell, which is a part of the ordinary somatic hypha. After the development of the conidiophore the overall structure becomes inverted T shaped.

The conidiophore is usually unbranched and aseptate. The tip of the conidiophore swells considerably, accumulates cytoplasm and forms a vesicle. The vertical part of the conidiophore keeps on elongating till it fully develops a spherical vesicle on its tip.

Several finger-shaped projections develop on the surface of the vesicle and they are known as **phialides** or **sterigmata**. There may be up to three layers of sterigmata on the vesicle. The first formed layer is known as primary sterigmata, and the subsequent layers as secondary and tertiary sterigmata. During the formation of sterigmata many thin areas develop in the vesicle wall due to the dissolution of wall materials. The vesicle cytoplasm is then pushed in these areas synchronously and thus finger-like outgrowths are formed.

Conidia develop in long basipetal chains on the sterigmata. There are 10-12 conidia in each chain. During the formation of a conidium, the nucleus of the sterigmata divides into two. Synchronously a globular protuberance develops at the tip of the sterigmata and it expands to form a conidium. One of the two daughter nuclei present in the sterigmata pass into the conidium. As the conidium expands, the wall of the sterigmata ruptures near its tip.

A long chain of conidia is formed in this fashion.

The conidium is a small, globose and uninucleate structure. It contains various pigments, such as yellow, green, brown or black, in different species. Conidia are dispersed by wind. They germinate on suitable substratum, each producing a new mycelium.

Sexual reproduction

Sexual reproduction is uncommon in *Aspergillus*. It has been studied in details in *A. flavus* and *A. repens*. Most species of *Aspergillus* are homothallic, but a few (e.g., *A. fischeri* and *A. heterothallicus*) are heterothallic. The male and female sex organs are known as antheridia and ascogonia (archicarp) respectively. The antheridium develops close to the ascogonium on the same or nearby hypha. The sexual process is achieved by **gametangial contact** between the trichogyne of the ascogonium and the antheridium.

There is a gradual elimination of antheridia in the some species of *Aspergillus*. The following stages of progressive degeneration can be seen.

1. In species like *A. herbanorum* antheridia are well developed and functional.
2. In *A. repens* and some other related species though antheridia are well developed, their cytoplasmic contents are not transferred to the ascogonium.
3. In many species of *Aspergillus* though antheridia are formed, the male nuclei soon degenerate and there are no functional nuclei in mature antheridia.
4. In *A. flavus*, *A. fischeri* and *A. fumigatus* antheridia do not develop at all.

After gametangial contact:

1. Ascogenous hyphae are formed
2. Ascus arises by Crozier process
3. Karyogamy takes place
4. Meiosis takes place
5. Spores are differentiated

Simultaneous to ascospore formation, the fruiting body, Cleistothecium also develops by mycelial aggregation.

Economic & Research Importance

Species of *Aspergillus* are without a doubt an important microorganism, both commercially and in biological researches.

1. In Asian countries, alcoholic beverages such as Japanese sake are made from rice, where *koji* mold such as *Aspergillus oryzae* is used to convert the starch in the rice to sugars (saccharification), which are subsequently fermented by other microorganisms, such as yeast (*Saccharomyces*) and lactic acid bacteria.
2. *A. niger* is as the major source of citric acid; this organism accounts for over 99% of global citric acid production, or more than 4.5 million tonnes per annum.
3. Various strains of *A. niger* are used in the industrial preparation of gluconic acid.
4. *A. niger* is also commonly used for the production of native and foreign enzymes, including glucose oxidase and hen egg white lysozyme.
5. Many therapeutically used enzymes are produced using *A. niger* including glucoamylase and α -galactosidase, which prevent flatulence.
6. Another use for *A. niger* within the biotechnology industry is in the production of magnetic isotope-containing variants of biological macromolecules for NMR analysis.
7. *A. nidulans* has been used as a research organism for many years and was used by Guido Pontecorvo to demonstrate parasexuality in fungi.
8. Recently, *A. nidulans* had its genome sequenced by researchers at the Broad Institute, USA.

Diseases caused by *Aspergillus* sp.

A number of species are parasitic and may cause diseases. Examples are:

1. If large amounts of *Aspergillus* spores are inhaled, a serious lung disease aspergillosis can occur. Aspergillosis is particularly frequent among horticultural workers breathing in peat dust which can be rich in *Aspergillus* spores.
2. *A. niger* causes otomycosis (fungal ear infections), which can cause pain, temporary hearing loss and in severe cases damage to the ear canal and tympanic membrane.
3. *Aspergillus fumigatus* and *Aspergillus flavus* cause invasive diseases in humans.
4. *Aspergillus* spp. cause disease on many grain crops, especially maize
5. *Aspergillus flavus* synthesizes a mycotoxin called aflatoxin, which causes cancer.

Neurospora

Neurospora is a genus of ascomycetous fungi. The genus name, meaning "nerve spore" refers to the characteristic striations on the spores that resemble axons. The best known species in this genus is *Neurospora crassa*, which is used as a model organism in biology.

The systematic position of *Neurospora*, under the new Fungal systematics, is given below.

Kingdom: Fungi
Phylum: Ascomycota
Class: Ascomycetes
Order: Sordariales
Family: Sordariaceae
Genus: *Neurospora*

The well studied species are:

N. crassa
N. intermedia
N. sitophila (also known as the Red Bakery Mold or Red Bread Mold)
N. tetrasperma

Life Cycle

In the somatic stage, the construction of the fungus is mycelia. The mycelium is well branched, multicellular and septate. The cells are multinucleate and contain pigments of various colours. The hyphae usually grow superficially on the substratum. The somatic stage is haploid.

Neurospora reproduces both by asexual and sexual means. In most species, asexual mode is the most favoured one and under the conditions of sufficient nutrition, all the species reproduce asexually. Sexual reproduction is triggered by the onset of unfavourable conditions, especially rising temperature and nutrient depletion in the substratum.

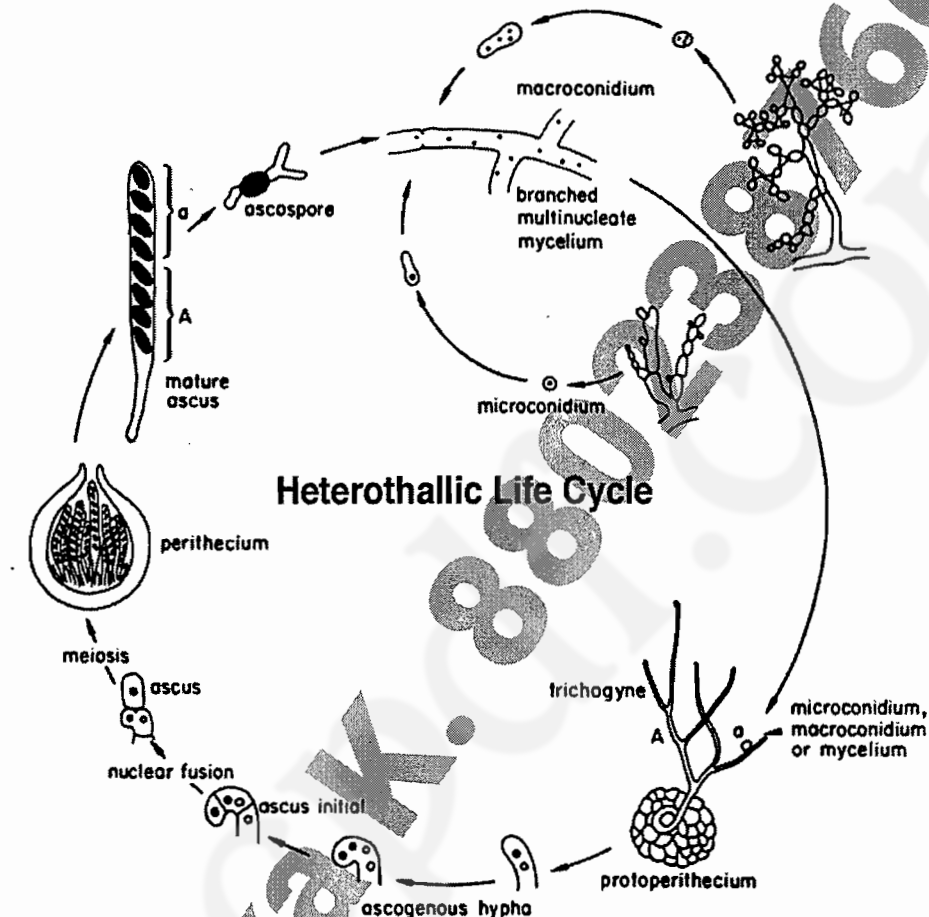


Figure 1: Heterothallic Life Cycle seen in *Neurospora crassa*. The upper right part of the figure is showing the Asexual process by means of Macro- and Microconidia. The rest of the figure shows the Sexual process with special reference to *N. crassa* (where Heterothallism is seen).

Asexual Reproduction

The formation of conidia is a common asexual method of rapid multiplication of *Neurospora*. The two types of conidia formed are *macroconidia* and *microconidia*.

1. **Macroconidia.** These conidia are large, oval and multinucleate, mostly pink in colour. They develop on erect aerial branches of the mycelium, known as *macroconidiophores*. The branched conidiophores produce conidia in beaded chains at the tips of their branches. The terminal conidium of a chain may further produce more conidia by budding. Conidia are dispersed by wind. The macroconidia belong to the form-genus *Monilia*. It means that earlier the macroconidia forming asexual stage was described under the form-genus name of *Monilia*.
2. **Microconidia.** The microconidia are also formed on erect aerial branches, called *microconidiophores*. These conidia are uninucleate, sticky and comparatively small than the macroconidia. They are borne in groups in terminal or lateral positions on conidiophores.

Both, macro- and microconidia form new mycelium on germination.

Sexual Reproduction

The various species of *Neurospora* show one of three different life cycles called *heterothallic*, *homothallic* or *pseudohomothallic*. The heterothallic *N. crassa* and *N. sitophila* are the most thoroughly studied species; their general cycle is shown in Fig. 1. The homothallic species of *Neurospora* include *N. teiricola*; *N. dodgei*.

N. tetrasperma is the best studied example of a pseudohomothallic species.

Sex organs. The female sex organs, known as ascogonia, develop as lateral outgrowths on the vegetative hyphae. The young ascogonium is a coiled, multinucleate and aseptate structure but septa are formed in the later stages of development.

Some hyphae develop from the base of the ascogonium and they form a pseudoparenchymatous ball-like structure around the ascogonium. The upper cells of the ascogonium give rise to long tapering *trichogyne*. The female sex organs of *Neurospora* are also known as *protoperithecia* or *bulbils*.

Antheridia are absent in *Neurospora*, but both macro- and microconidia may act as *spermata*.

Plasmogamy. At the time of fertilization, micro- or macroconidia (which act as *spermata*) get adhered to the trichogyne. The walls between the trichogyne and the fusing conidium dissolve at the point of their contact and one or more nuclei from the conidium enter the ascogonium through the trichogyne. In some species of *Neurospora*, plasmogamy occurs through somatogamous copulation.

1. In **heterothallic species**, conidia of one strain unite with the trichogyne of the ascogonium of another strain (Fig 2). Heterothallic means that there are two mating types that must unite to be able to go through the sexual cycle. The two mating types look identical. The two mating types are determined by alternative DNA sequences at one chromosomal locus; these are called *MAT A* and *MAT a*. These two mating type sequences are totally different, so they do not represent alleles; instead they are called *idiomorphs*. *N. crassa* is the most analyzed heterothallic species.

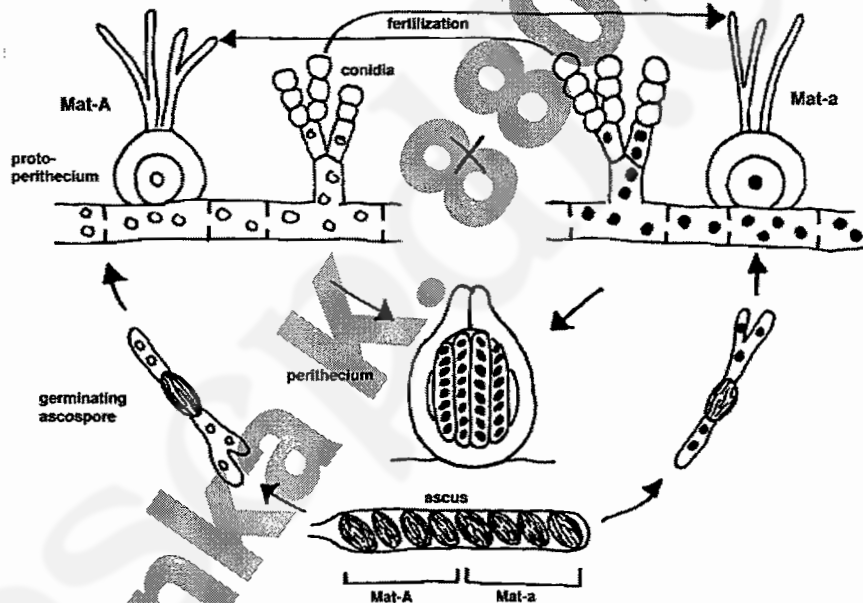


Figure 2: Heterothallism in *N. crassa*

2. In **homothallic species** the conidia of the same strain fuses with the trichogyne of a given strain. Homothallic means that any haploid individual strain can go through the sexual cycle by itself, without pairing with another strain. Homothallic species do not need both *MAT* idiomorph sequences. *Neurospora galapagoensis*, *N. teiricola*; and *N. dodgei* are homothallic species. (Fig. 3)

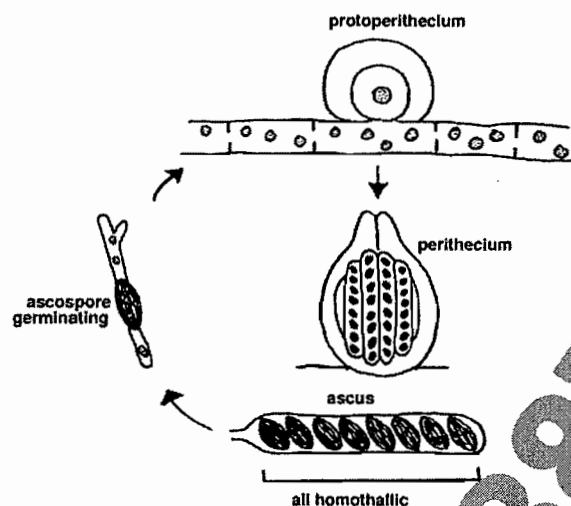


Figure 3: Homothallic reproduction in *Neurospora*

3. **Pseudohomothallic** species also require both *MAT* idiomorphs to complete the sexual cycle, thus in this sense they act as heterothallic species. However, the nuclei that result from meiosis are packaged in such a way that one *MAT A* and one *MAT a* nucleus is included in any ascospore. Hence an individual spore will grow into a dual mating type hypha, which contains both the nuclei necessary to go through the sexual cycle like homothallic individuals and need not pair up with any other individual to do so. *N. tetrasperma* is the best studied example of a pseudohomothallic species.

Development of ascus. Shortly after plasmogamy the ascogonium develops one or more ascogenous hyphae. Each cell of the ascogenous hypha contains a dikaryon. The terminal cell of the ascogenous hypha is curved and forms a hook-like structure, known as *crozier*. Both the nuclei of crozier show conjugate division (i.e., both nuclei of a dikaryon divide simultaneously) to form four daughter nuclei. Thereafter, by the septation of crozier, three cells are formed in which the nuclei are so distributed that curved terminal cell has one, penultimate cell two (dikaryon) and the basal cell one daughter nuclei. The two nuclei of the penultimate cell fuse to form a diploid nucleus. This cell acts as ascus mother cell and it elongates to form a club-shaped or cylindrical ascus. The diploid nucleus of the ascus first divides meiotically and then mitotically, resulting in the formation of eight haploid nuclei. Each nucleus secretes a wall around itself and metamorphoses into an ascospore.

In *N. tetrasperma*, only four ascospores are formed in an ascus. The ascospores are arranged in a single row in the ascus. They are dark brown or black in colour and have characteristically ribbed wall. The globose envelope of sterile hyphae encloses the maturing ascogonium from all sides, resulting in the formation of young perithecium.

The mature perithecium is a dark-coloured, globose, flask-shaped and beaked structure, which remains surrounded by dark pseudoparenchymatous peridium. The canal of the neck is lined by periphysis. Paraphyses are also present between the asci in mature perithecium. The asci are cylindrical, long and stalked.

Ascospores germinate to produce new mycelium. They remain viable for many years in *N. crassa*. Ascospores can be readily germinated by chemical (e.g., furfural) or high temperature (60° C for 20 mm.) treatment.

Importance of *Neurospora* in biological studies

1. *N. crassa* is used as a model organism because it is easy to grow and has a haploid life cycle that makes genetic analysis simple since recessive traits will show up in the offspring. For this reason, *Neurospora* is also called the *Drosophila* of the botanical world.
2. *Neurospora* was used by George Wells Beadle and Edward Lawrie Tatum in mutation experiments in order to discover mutants that would differ in nutritional requirements. The results of their experiments led them to hypothesize upon the one gene-one enzyme hypothesis, in which they postulated that every enzyme was encoded with its own gene.
3. *N. crassa* has been widely used in linkage analysis by Tetrad analysis method.
4. *N. crassa* has also been a model organism to study fungal metabolism due to its simple and flexible nutritive requirements.

5. In the 24 April 2003 issue of *Nature*, the genome of *N. crassa* was reported as completely sequenced. The genome is about 43 megabases long and includes approximately 10,000 genes. There is a project underway to produce strains containing knockout mutants of every *N. crassa* gene.

Claviceps

This fungus from the Class Pyrenomycetes of Phylum Ascomycota is a plant parasite, commonly found on grains of rye or sometimes on other grasses belonging to the family Poaceae.

Species of this genus infect many wild and cultivated hosts of Poaceae. From India, about eight species have been reported, of which *Claviceps purpurea* and *C. fusiformis* are most common. The former infects jowar, sugarcane, *Andropogon*, *Cynodon* etc., whereas the latter bajra in U.P., Bihar, Mysore and Maharashtra states. Both species cause the ergot disease in these plants. *C. purpurea*, the cause of ergot of grasses and cereals grows on a wide range of grasses. Grasses and cereals infected with this species develop purple curved sclerotia (ergots) in place of healthy grains.

It is not a devastating parasite to the plant. Its main detriment is that it replaces one of the grains of the plant, thus reducing yield by 5% to 10%. However the sclerotia left behind in the inflorescence is a frequent cause of food poisoning.

Primary Infection & Asexual Phase

The fungus infects the flowers when they are young. Primary infection of plant occurs by *ascospores* released from the perithecia. The ascospores settle on the feathery stigma of the host flower and germinate. Each spore puts forth several germ tubes. The germ tubes cross the stylar canal, reach the ovary and penetrate the ovary wall to grow vigorously to form the mycelium. The hyphae are branched and septate with hyaline cells. Finally the ovule is occupied by a mass of fungus hyphae.

After about a week of primary infection, some hyphae from the mycelial mat in the host ovary, grow outwards through the ovary wall. The exposed tips of these hyphae function as the *conidiophores*. The numerous, short conidiophores spread over the entire surface of the ovary in a dense columnar way. Small, unicellular ovate conidia are budded by each conidiophore. Earlier mycologists had described these conidia under the name *Sphacelia segetum*. Thus, the conidial stage came to be known as the *Sphacelia* stage of this fungus. But later it was realized that these belonged to *C. purpurea*.

The conidiospores are enveloped in a sticky, sweet liquid called the *honeydew* which being rich in glucose, fructose, sucrose etc attracts the insects. The insects serve as the vector for conidiospore dispersal. The conidia infect healthy young ovaries secondarily.

Later, towards the end of the host's growth season, the *sphacelia* convert into a hard dry *sclerotium* inside the husk of the floret. The sclerotia lie between the glumes of the spikelet, generally protruding out of glumes as a dark spur on the spike. Various alkaloids and lipids accumulate in the sclerotium.

The sclerotia are hard resting structures that allow the fungus to survive adverse conditions, such as winter and desiccation. In the life cycle of this organism, the sclerotia fall to the ground and overwinter, germinating in the spring to produce a stroma that contains perithecia, which contains the sex organs and later the asci.

The sclerotia are also known as *Ergot*, since it contains the Ergotamine family of alkaloids. Most of these compounds are related to of Lysergic Acid [LSD], as a result *Claviceps* is also a source of this hallucinogenic drug.

These compounds include:

1. Ergotamine,
2. Ergonovin,
3. Ergometrin,
4. Ergosine,
5. Ergonine etc.

As mentioned earlier, the sclerotia left behind in the inflorescence is a frequent cause of food poisoning resulting into a devastating and sometimes deadly syndrome called *ergotism* in humans and other animals. Consumption of foods contaminated with ergot and ergot derivatives causes vomiting, diarrhea, burning sensation in muscles and eyes, muscular convulsions, hallucinations, and may even lead to gangrene in serious cases. Historically the fungus has been implicated in epidemics causing thousands of fatalities, but due to increased knowledge of this fungus and a more varied modern diet such epidemics no longer occur in humans. However, chronic exposure through consumption of contaminated foods can lead to health complications.

These chemical prevent the consumption of the sclerotia by the mycophages.

There are medicinal products also that have been extracted from Ergots. Some of the more common example include **ergotamine**, prescribed for various causes of headaches. **Ergonovine** is used to control postpartum hemorrhage and cause contraction of the uterus. The knowledge that the ergot could be used for causing contraction of the uterus has extensively been applied earlier to facilitate child birth. However, due to undesirable effects, the application of ergot derivatives in obstetric practices has been stopped.

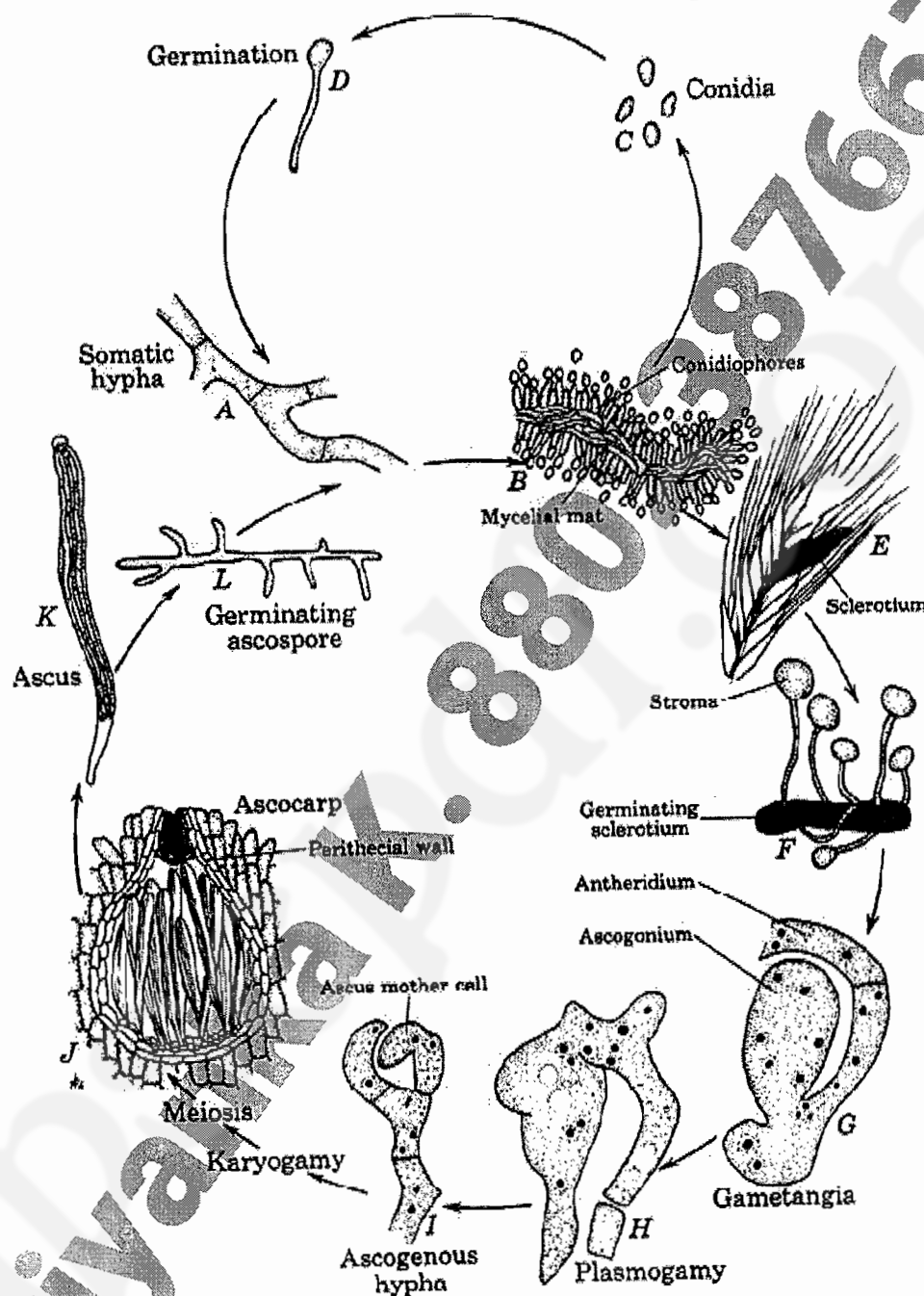


Figure 1: Life Cycle Sketch of *Claviceps purpurea*

Sexual Phase

It begins with sclerotia germination in the next favourable period, which results into a number of small pink or dark purple, drum-stick like structures, called *perithecial stromata*. These stromata are 1-2 cm in length, positively phototropic and are differentiated into stalk and *capitate head*. There are many minute cavities in the stromatal head. They are known as *perithecial cavities*, and are surrounded by pseudoparenchymatous tissue.

Both, male (antheridium) and female (ascogonium) sex organs are present in a perithecial cavity. The sex organs develop from the terminal cells of the hyphae present at the base of the cavity. The cytoplasm of the fertile hypha is comparatively dense than that of the somatic hyphae. Both, antheridium and ascogonium are multinucleate at maturity. The ascogonium is usually stouter and broader than the slender and elongated antheridium.

As the ascogonium matures, a small papilla-like outgrowth appears on its lateral side. This outgrowth comes in contact of the neighbouring antheridium. The walls of the ascogonium and antheridium dissolve at the point of their contact and plasmogamy takes place. In the process, male nuclei from the antheridium migrate into the ascogonium.

Following plasmogamy, numerous ascogenous hyphae are produced from the base of the ascogonium. The penultimate dikaryotic cell of an ascogenous hypha functions as ascus mother cell. The two nuclei of this cell fuse to form a diploid nucleus. It becomes narrow and tubular by elongation and forms an ascus. The diploid nucleus of the ascus divides first meiotically and then mitotically to form eight haploid nuclei. Each nucleus transforms into an ascospore by accumulating some cytoplasm and developing a wall around itself. The ascospores are elongated, hyaline and thread-like structures. All the eight ascospores lie parallel to one another in an ascus. A conspicuous cap is present at the tip of the ascus. The perithecial walls grow around the developing asci within the stromatal heads. The whole structure thus formed is called fruiting body or ascocarp. Ascospores are discharged forcibly due to hygroscopic pressure and are ejected one by one from the ascus. Soon after their release, they are disseminated by wind. They are carried to the flowers of healthy host plants, where they germinate and infect the ovaries

Peziza

Introduction

The order Pezizales contains the operculate discomycetes which are the most readily recognized cup fungi. The order is large, containing some 15 families, about 160 genera and over 1100 species (Kirk et al., 2001). Most are terrestrial and saprotrophic on soil, burnt ground, decaying wood, compost or dung, but some form sheathing mycorrhiza (ectomycorrhiza) with trees.

Species of *Peziza*, commonly known as cup-fungi, grow as obligate saprophytes on animal dung, decaying wood, manure piles, wide range of domestic materials including plaster, cement, sand, coal dust, wet rugs & carpets, fire-place ashes, walls, and on the soils rich in humus. The genus includes about 150 species, out of which 25 species have been reported in India. The two most common Indian species are *P. ecbinospora* and *P. mutiguttulata*. The genus can be recognized on the basis of large cup shaped ascocarps-the sexually produced fruiting bodies, called **apothecia** that develop from the mycelium growing on or within the substrata such as decaying wood, animal dung, and soil rich in humus

Structure

The vegetative thallus of species of *Peziza* consists of perennial mycelium which is profusely branched and represents septate hyphae that ramify the substratum on which it is growing. The septate hyphae have uninucleate cells. The ramifying hyphae within/on the substratum extract organic and inorganic nutrients from the substratum. The fungus survives in the substratum with the help of dormant mycelium or due to formation of thick-walled **chlamydospores**. The chlamydospores are produced singly or in series with in the cells of the mycelium. Each such chlamydospore under the favourable conditions, germinates to produce a new mycelium. The aerial hyphae are represented by conidiophores and, the cup or disc-shaped sexually produced ascocarps are also produced aurally on the surface of substratum in the form of **Apothecia**.

Reproduction

It reproduces asexually and sexually

Asexual reproduction

Asexual reproduction is rare and takes place by means of conidia.

The conidia are unicellular hyaline or lightly coloured and elliptical, and on dispersal through wind or water, germinate to produce, branched septate mycelium on or within the substratum.

Sexual reproduction

No sex-organs are known in *Peziza*. The vegetative hyphae grow in all directions to give a pseudoparenchymatous mass. Within this hyphal

mass, there may be seen a weft of branching filaments which are fairly more rich in protoplasmic contents. Each cell of the weft contains one or

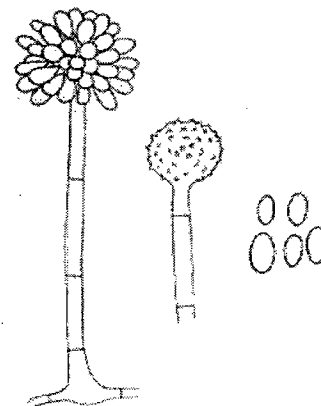


Figure 1: Conidia in *Peziza*

more nuclei. The nuclei have been seen to migrate from one cell of the web to another. Thus, there is a **somatogamous copulation** between vegetative cells of these filaments. Nuclei in such cells then arrange themselves in pairs-dikaryons.

In some cases dikaryotic condition may also be achieved by autogamous pairing i.e. the nuclei of the same cell arrange themselves in pairs.

The cells of these dikaryotic hyphae give rise to ascogenous hyphae, and the asci are formed on them in usual manner. Karyogamy and meiosis occur in the enlarged terminal cells of ascogenous hyphae which behave as ascus mother cells. The croziers have not been observed. Each ascus mother cell ultimately produces a cylindrical ascus having eight linearly arranged oval or elliptical ascospores.

Ascocarp in *Peziza*

In *Peziza* sp. the ascocarp is an apothecium.

The apothecia are cup-shaped, often large (25 cm or more), usually pale brown and fleshy. They are commonly encountered in a very wide range of habitats including soil, manure heaps, dung, rotting wood or straw, burnt ground and sand dunes. The apothecia grow above ground but about six species have hypogaeous asco carps (Trappe, 1979).

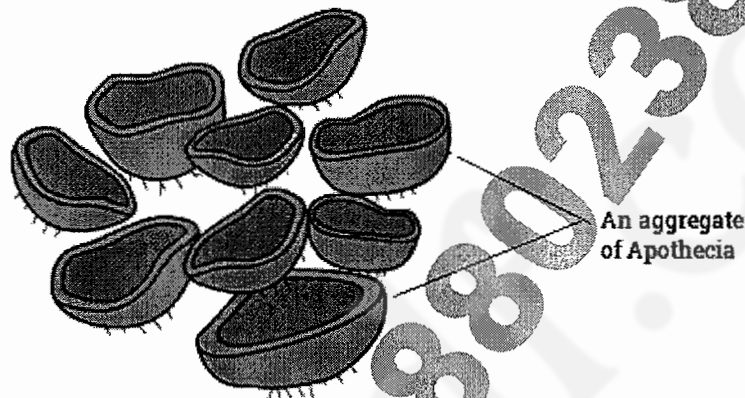


Figure 2: An aggregate of Apothecia in *Peziza*

If vertical section of an apothecium is examined microscopically, it can be divided into **hymenium**, **subhymenium**, and the fleshy pseudoparenchymatous tissue called **excipulum**. (Fig. 3)

The excipulum is further differentiated into outermost, one-layered epidermis called **ectal-excipulum**, and inner multilayered **endal-excipulum**, representing multilayered pseudoparenchymatous tissue, called **medullary excipulum**.

In the top most layer, that is, **hymenium**, the asci stand side by side intermingled with the paraphyses. The loosely interwoven hyphae below the hymenium, constitute the subhymenium or **hypothecium**. The **excipulum** constitutes the larger part of the mature cup-or saucer shaped ascocarp, the **apothecium**. In the edible species, this pseudoparenchymatous tissue is the main delicious part rich in **proteins**. On dry weight basis, proteins represent nearly 75% of the total edible component.

The main reproductive or fertile part of the apothecium is represented by **hymenium**. It contains cylindrical asci each containing eight linearly arranged ascospores, intermingled with long sterile hyphae called paraphyses, having swollen tips emerging on the surface of the hymenium. The swollen tips of the paraphyses are at high turgor pressure, and form an epidermis-like layer called **epithecium**, at the surface of hymenium as the swollen tips are held together very tightly. The cell-sap in the swollen tips of paraphyses representing epithecium, is at a very high osmotic pressure. The epithecium, thus, acts as a barrier and protects the inner tissues of apothecium from invasion by the growth of microflora and microfauna that might penetrate the swollen tips of paraphyses to feed on the rich protoplasmic components.

When the ascospores are mature, the maturing asci from below push in between the paraphyses, and liberate their ascospores to be dispersed by wind. The empty asci, finally collapse.

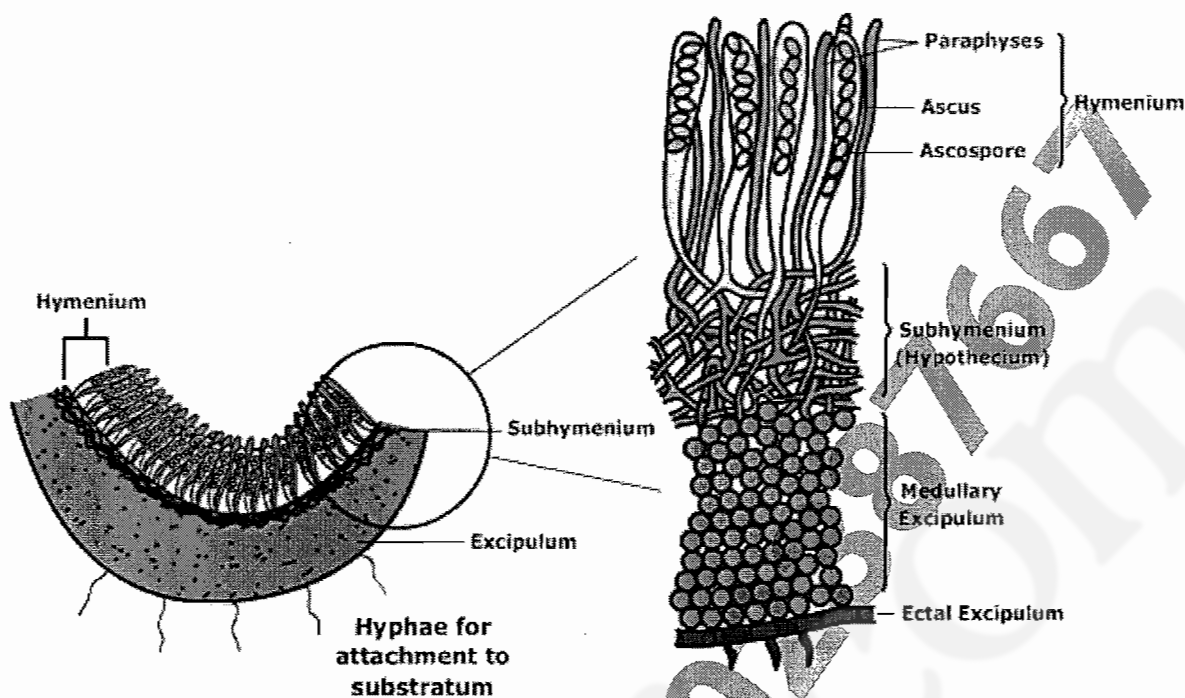


Figure 3: Structure of *Peziza* apothecium

Economic importance of *Peziza*

1. **As Decomposers of Organic Matter:** Since all the species of *Peziza* are obligate saprophytes, they have the potentiality to grow on all kind of organic substrata. *P.domiciliana* is known to spoil domestic materials, especially coal dust, wetrugs and carpets, fire place ashes as well as walls of kitchen and old constructions. *P. phyllogena*, *P.repanda*, *P.vesiculosa* and *P.ampliata* act as common decomposers of old wood chips and rotten woods. *P.domiciliana* has been reported as responsible for degradation of construction wood used in historical monuments in Moldavia.
2. **As Food and Health Hazard:** Of all the species of *Peziza*, *P.domiciliana* is being used as food in the Eastern Himalayas, Europe, North America and Antartica. There are in general, no reports of adverse health effects or toxicity in people who have been using apothecia of *P.domiciliana* as food. However, this fungus has been known to promote *pneumonitis* in a previously healthy woman who developed severe *dyspnea* leading to *alveolitis* after regularly consuming the cups of this fungus as food.

Basidiomycota

Introduction to the Basidiomycota

Basidiomycota is the most advanced phylum of true fungi, which make the Kingdom Mycota. It is also the second largest true fungal phylum after Ascomycota. It shares many features with Ascomycota. For this reason, the two phyla are also called the *sister phyla of Fungi*.

The phylum includes many economically and ecologically important genera. Some important members include mushrooms, puffballs, stinkhorns, bracket fungi etc.

The important features of this phylum are as follows.

1. Most members are saprobic. A few are parasitic and some form mycorrhizal associations with tree roots.
2. All members are characterized by the formation of *basidiospores*. These are haploid spores formed by meiosis in the zygote. They are exogenously formed on a club-shaped hyphal segment called *basidium*.
3. In most cases, the members have mycelia organization (except for those forming unicells). Thus, the structural units are hyphae.
4. The hyphal wall has large amounts of chitin.
5. The hyphae are septated with a single or more than one central perforation. The perforation may be simple or more complex *Dolipore type*.
6. The hyphae remain dikaryotic for a significant part of the life cycle. This is also seen in members of Ascomycota. For this reason, the Ascomycota and Basidiomycota together comprise the subkingdom Dikarya within the Kingdom Fungi.
7. The nucleus in the trophic stage is haploid.
8. Reproduction involves both asexual sporulation and sexual processes. In sexual process, plasmogamy establishes a dikaryotic phase, which is usually quite long.
9. Karyogamy occurs just before basidiospore formation. It is immediately followed by meiosis.

Reproduction in Basidiomycota

This group of fungi is characterized by the most complex and large structures found in the fungi.

They are also very advanced fungi and they mostly reproduce by sexual means. They very rarely produce asexual spores.

Most of the life cycle is spent as vegetative mycelium, feeding upon complex substrates. Sexual process is initiated after acquisition of certain hyphal growth.

As most species are heterothallic, a preliminary requisite for sexual reproduction is the presence of hyphae of two mating types. Only such hyphae are compatible. Plasmogamy brings both the types of nuclei in the same cell. This cell is now called *heterodikaryon*. This is a long lasting stage in Basidiomycota. Its maintenance requires elaborate septum formation by *clamp connections* during growth and nuclear division.

Onset of sexual-spore formation is triggered by environmental conditions. It begins with the formation of a fruit body primordium. Dikaryotic mycelium expands and differentiates to form the large fruit bodies or *basidiocarps*. Basidiocarps are not formed in members of *Teliomycetes*.

In special hyphal segments, the karyogamy occurs. This step is called *Diploid formation*. After this meiosis occurs within those modified hyphal tips where karyogamy has taken place. This modified hyphal tip is now called a *basidium* (Fig.1).

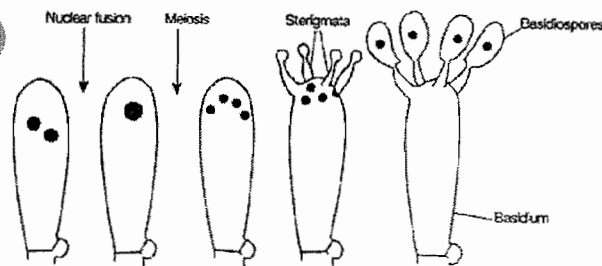


Figure 1: Basidiospore formation in Basidiomycota

Four spores are budded from the basidium. Basidia form together to create a hymenium.

The hymenium is highly sensitive to the presence of free water. The hymenium is distributed over sterile, dikaryotic supporting tissues which protect it from rain.

Similarities with Ascomycota

1. Hyphal structure
2. Chitinous wall
3. Hyphal anastomosis and formation of complex structures
4. Primary septum present within the hyphae
5. Primary septum is perforated
6. Trophic stage nucleus is haploid
7. Asexual reproduction by conidia
8. No production of motile cell at any stage of life cycle
9. A long gap between plasmogamy and karyogamy
10. Plasmogamy establishes a prolonged dikaryotic stage
11. Most members produce elaborate fruiting bodies

Puccinia : Life Cycle

Puccinia (after T. Puccini, Italian anatomist) is the largest genus of Fungi with more than 4,000 species, parasitizing angiospermic plants throughout the world. One of the important features is that the *teliospores* are 2-celled (though in some species the spores are 1-celled - and in such cases they are called *mesospores*).

The species may be heteroecious or autoecious with a macrocyclic or microcyclic life cycle. Economically they are responsible for causing heavy loss in cereals and millets.

Life cycle of *P. graminis*

P. graminis, the cause of black stem rust of cereals and grasses is a long-cycled, heteroecious rust alternating between cereals and grasses on one hand and barberry (i.e. species of *Berberis* like *B. vulgaris*, *B. canadensis*, *B. cretica*) on the other. This species consists of several subspecies or specialized forms, specialized in their parasitism on different groups of cereal crops.

In *Puccinia graminis* (Wheat Rust) there are five spore stages that are produced and two hosts are required in the completion of the life cycle. The five stages produced are:

- Stage 0: Spermatogonium
- Stage I: Aecium
- Stage II: Uredium
- Stage III: Telium
- Stage IV: Basidium

This numerical system for different stages was given by Hiratsuka in 1973. They are depicted in Fig. 1.

Spermatogonium (Stage 0)

The spermatogonium stage produces the sex organs in rusts. They are produced on the upper surface of the *Berberis* (barberry) leaf. Since the spermatogonia are derived from basidiospores, they are of two mating types. They are flask-shaped and produce spore-like *spermatia* - which ooze out, from the neck, in a sweet-smelling nectar. Also growing from the necks are *receptive* or *flexuous hyphae*. The spermatogonia are visited by flies which are attracted by the nectar secretions, and as they visit different spermatogonia, spermatia of both mating types, adhere to their bodies and are transferred to receptive hyphae of the other mating types. This begins the dikaryon stage of the life cycle.

Aecium (Stage I)

The aecium stage is directly linked to the spermatogonium stage. When spermatia are transferred to compatible receptive hyphae, this begins the dikaryotic stage of the life cycle and directly produces the aecium on the lower surface of the barberry leaf. The aecium is an upside-down, sac-shaped structure in which chains of *aeciospores* are formed. The aeciospores burst through the lower surface of the leaf and are dispersed by wind.

Uredium (Stage II)

The aeciospores cannot reinfect the barberry host. Instead infection can only occur on *Triticum aestivum* (wheat), where a new dikaryotic infection occurs. When more than one hosts are required in the completion of a rust life cycle, the rust is said to be *heteroecious*. The dikaryotic aeciospores infect the wheat stems and leaves and form *uredia* that contain orange-brown *urediospores*. This order is commonly called the rusts because

of the orange-brown (rusty) colored pustules that form on the wheat plant after the uredia have broken through the epidermal surface. The urediospores are comparable to conidia in that they will reinfect wheat plants and produce more uredia and urediospores. In a given cropping season there can be 4 to 5 rounds of infection by urediospores.

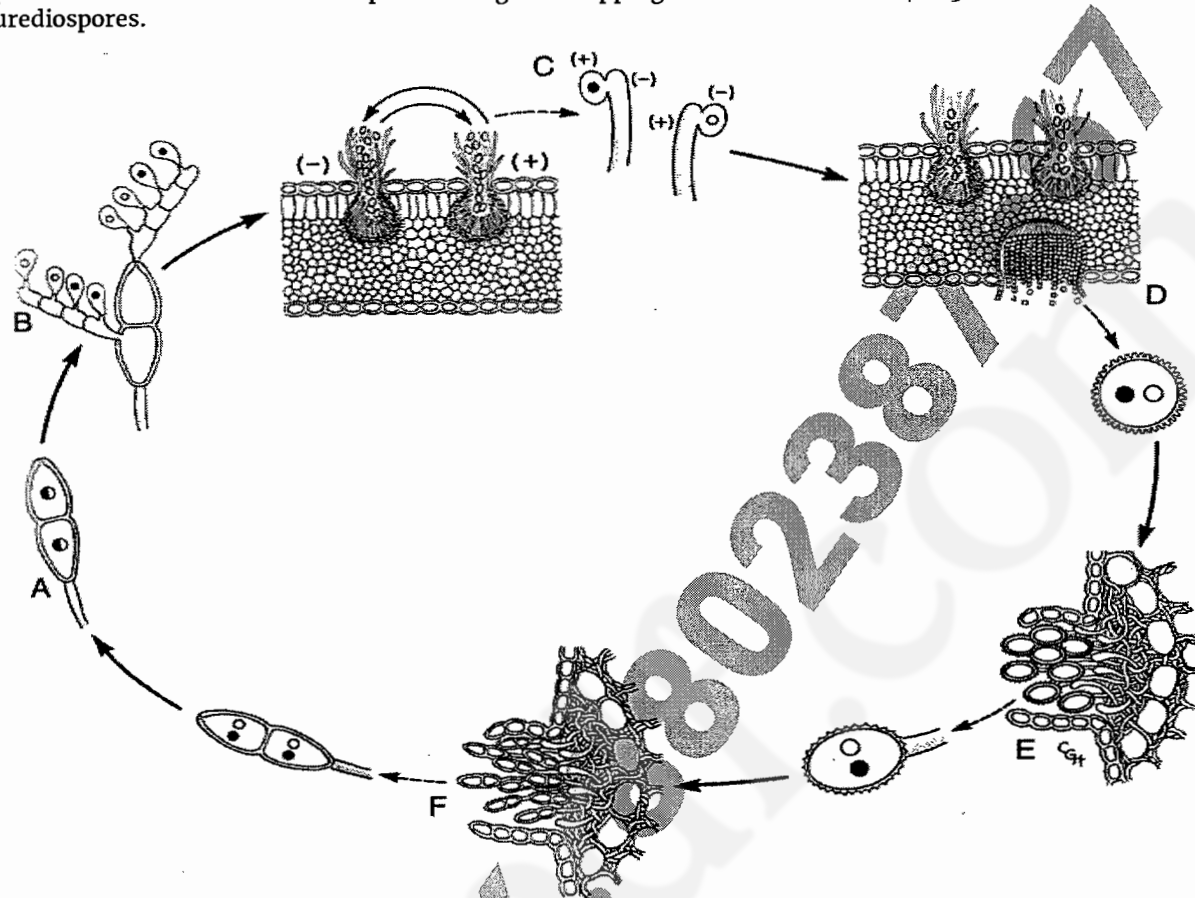


Figure 1: Life cycle of *Puccinia graminis*: (A) mature, diploid teliospore; (B) basidia with basidiospores; (C) spermogonial stage on barberry; (D) aecial stage on barberry; (E) uredial stage on wheat; (F) telial stage on Wheat.

Telium (Stage III)

Towards the end of summer, the uredium begins to produce *teliospores*, a dark, thick-walled, two celled spore. As the summer approaches, the entire uredium gradually becomes a telium and produces more and more teliospores. Because of the color of the teliospores, the telium is black.

Each cell of the teliospore initially has two nuclei each but later karyogamy occurs and then each cell of the teliospore contains a single diploid nucleus each. Following karyogamy, the teliospore overwinters. Meiosis takes place in each cell of the teliospore in spring and germinates to form the *promycelium* (= basidium). The promycelium becomes transversely septate, forming four cells. Each cell produce a sterigma and a basidiospore.

Basidium (Stage IV)

Puccinia graminis is heterothallic and *basidia* produce *basidiospores* that are of two mating types, designated as a1 [+] and a2 [—]. Basidiospores are capable of only infecting the leaves of *Berberis* sp. (barberry), the alternative host for this species. Species that require two hosts to complete their life cycles are said to be heteroecious. The cells of the teliospore germinate to produce a short germ tube that will develop into a basidium that is essentially transversely septate.

Control of *P. graminis*

Control of wheat rusts is possible by several means which can be classified into the following 4 categories.

1. Use of fungicides: Zinc or Manganese based *Dithiocarbamates*, available in our country, can check the disease provided farmers start spraying the fields with the first appearance of rust pustule and continue to give 3-4 sprays at regular intervals of 12 to 15 days, depending upon the location. Systemic fungicides such as Oxycarboxin (Plantavax) holds promise for future control.

2. Eradication of the alternate host. Eradication of the alternate host is helpful in controlling the disease in those countries where the alternate host is effective such as the U.S.A., Canada or France. In India the alternate host of black stem rust is non-functional (Mehta, 1952) and whatever species of barberry or *Mahonia* are in the hills, they are not susceptible to *P. graminis tritici* (black stem rust of wheat) and fortunately *Isopyrum fumarioides*, the alternate host of *P. recondita* does not occur in India.

3. Breeding for resistance. Breeding for disease resistance can be said to be the best method for controlling the rusts. Breeding for rust resistance in India began in 1935 when Dr. B. P. Pal and Late Dr. K. C. Mehta jointly approached the problem and since then extensive breeding programmes to transfer desirable characters along with high degree of resistance in the commercial wheat have been taken. With the introduction of Mexican varieties of wheat in our country black stem rust of wheat has declined—because these varieties are comparatively resistant to black stem rust of wheat. In recent years, a variety HD 2135 has been recommended for cultivation in the Nilgiri hills which is one of the main foci of infection of brown and black rust in India with the hope of cutting down the initial inoculums at its very sources.

4. Cultural practices. Cultural practices like judicious application of nitrogen fertilizers, mixed cropping, early maturing varieties are helpful in checking the disease.

Ustilago: Life Cycle

Ustilago is a large genus of Basidiomycota belonging to the order Ustilaginales and includes about 510 species, most of which are parasitic on a large number of crop plants like wheat, rice barley, oat, maize, jowar, sugarcane etc. causing a group of diseases known generally as the *smuts* (Fig. 1). They are called smuts because of their black coloured spores, formed within the tissues of the host plants which give the infected part a burned or charred appearance.

They are very important on account of the fact that a considerable number of species cause plant diseases on cereal crops of serious economic importance. Unlike the rusts, they are not obligate parasites, since many of them have been grown on synthetic media, an indication that they are capable of saprophytic growth. Butler and Bisby (1958) reported 10 species of *Ustilago* from India. The smut diseases are common in all parts of India. In U.P. the smut disease is commonly known as *Kandua* and in Punjab it is called *Kangiar*.

Following are the different species of *Ustilago* which cause various important diseases of crops (Numbering as shown in Fig. 1)

- A. *U. nuda* – Loose smut of barley.
- B. *U. cyanodontis* – Smut of wild grass
- C. *U. hordei* – Covered smut of barley.
- D. *U. kolleri* – Covered smut of oat.
- E. *U. maydis* – Smut of maize
- F. *U. scitaminae* – Whip smut of sugarcane.

Not shown in the picture:

- G. *U. avenae* – Loose smut of oats.
- H. *U. tritici* – Loose smut of wheat.

Somatic & Asexual phase

Depending upon the nuclear behaviour, the

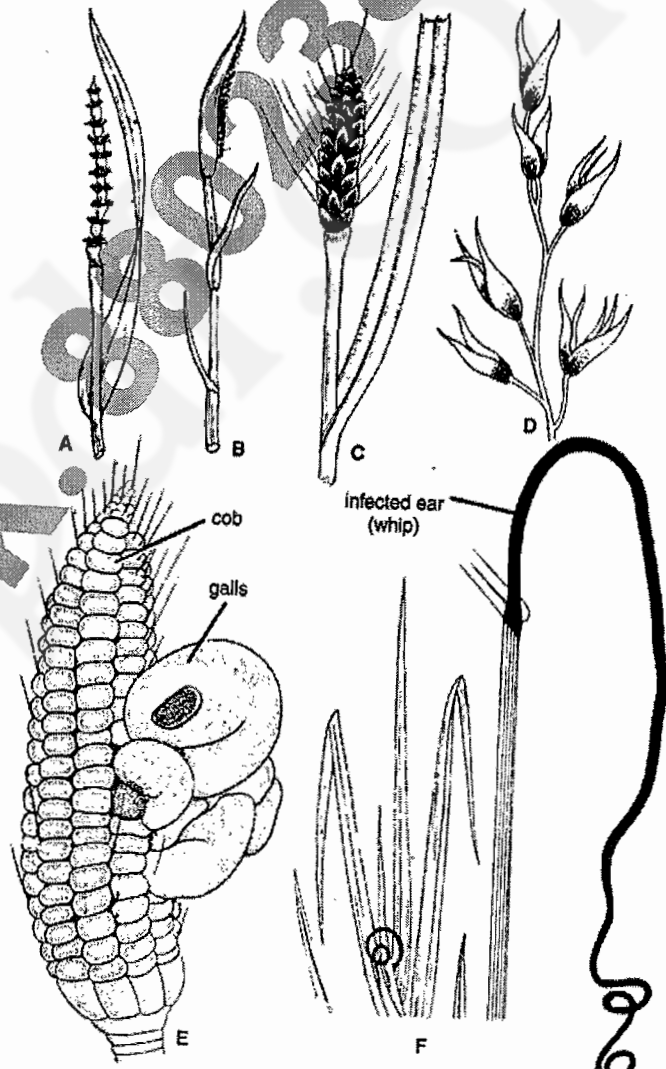


Figure 1: The smut diseases common in India. A) *U. nuda* – Loose smut of barley; B) *U. cyanodontis* – Smut of wild grass; C) *U. hordei* – Covered smut of barley; D) *U. kolleri* – Covered smut of oat; E) *U. maydis* – Smut of maize; F) *U. scitaminae* – Whip smut of sugarcane.

somatic body of *Ustilago* passes through two distinct stages of development. These are the *primary* and *secondary* mycelia.

The primary mycelium is uninucleate. This kind of mycelium develops by the germination of a basidiospore and is also called *monokaryotic mycelium* or *haplomycelium*. This kind of mycelium is of short duration, for it soon becomes diploidised or dikaryotised to produce secondary mycelium.

The secondary mycelium, which constitutes the most conspicuous and important part of the vegetative phase, is composed of septate and extensively branched, dikaryotic hyphae. The secondary mycelium is at first intracellular and later becomes intercellular, with or without haustoria. Haustoria are present in *U. avenae* and *U. nuda*.

The secondary mycelium which is composed of binucleate (n+n) cells, spreads through the body of the host plant. Though the infection occurs at the seedling stage of the host, it has little or no effect on vegetative development of the host. At the time of sporulation the hyphae form dense tangled masses in certain portions of host such as inflorescence, leaves and stems. The cells of this mycelium become transformed into a mass of black, thick-walled chlamydospores. These spores are also called the *brand spores* or the *teleutospores* or the *smut spores*. They are spherical and binucleate structures.

The first visible symptoms of the disease become evident only at the flowering stage.

Two types of smuts are recognized namely the *loose smuts* and the *covered smuts*. In loose smuts, e.g., *U. tritici* on wheat and *U. nuda* on barley, the spikelets are converted into large, dusty sori and the powdery masses of spores are exposed. In the covered smuts, e.g. *U. hordei* (Sumt of barley), the smut spores remain in the grain and are only freed during the threshing of the grain. In this case, the fungus does not totally destroy the ovary wall of the host flower.

The germination of teleutospores may occur immediately or following a dormancy period. The smut spores that are carried by wind may fall on the soil, on the grain, and other favourable places, leading a saprophytic existence. Under suitable conditions such as warmth and moisture, they germinate.

Sexual phase

Sexual phase has two events:

1. Plasmogamy
2. Karyogamy

Plasmogamy occurs usually very early in the life cycle of this fungus. When the basidiospore germinates, each basidiospore gives rise to a fine germ tube which is *monokaryotic*. In most species, a monokaryotic germ tube cannot infect the host tissues excepting *U. maydis*.

The infection is established only when the diploidization has already taken place between the cells of the germ tubes. In *U. maydis*, the monokaryotic germ tube infects the host and dikaryotization subsequently takes place within the host.

Dikaryotization by plasmogamy may take place outside or inside the host tissue and may occur by means of following methods:

1. by fusion between the two hyphae from the primary mycelia of opposite strains, e.g., *U. maydis*,
2. by fusion between the germ tubes of two basidiospores of opposite strains, e.g., *U. hordei*,
3. by conjugation between the two secondary basidiospores of opposite strains that are produced by the budding of the basidiospores or between two primary hyphae,
4. by the union of the basidiospore of one strain with the germ tube of opposite basidiospore,
5. by the union of two infection threads, e.g., *U. tritici*, and
6. by the fusion between two basidia formed by the germination of smut spores of opposite strains, e.g., *U. nuda*.

The dikaryon phase is quite long lasting and as a matter of fact, in most of the species the somatic phase is actually a dikaryon, which is known as the secondary mycelium.

As already mentioned, the secondary mycelium (n+n) spreads through the body of the host plant. At the time of sporulation the secondary hyphae form dense tangled masses in certain portions of host such as inflorescence, leaves and stems. The cells of this mycelium become transformed into a mass of black, thick-walled chlamydospores. These spores are also called the *brand spores* or the *teleutospores* or the *smut spores*. They are spherical and binucleate structures.

The germination of teleutospores may occur immediately or following a dormancy period. When the smut spores germinate, the exosporium or the episporium layer of the spores becomes ruptured. The endospore or the endosporium protrudes out in the form of a cylindrical hypha, the *Promycelium* or *epibasidium*, into which the diploid nucleus migrates. Meiosis occurs and the four haploid nuclei are formed in the epibasidium. The epibasidium converts into a *phragmobasidium* and bears four basidiospores.

In heterothallic species, such as *U. maydis*, the segregation into plus and minus strains takes place during the meiosis before the formation of the basidiospores.

In some species e.g., *U. maydis*, the basidiospores after abstriction bud like yeast cells. The new spores formed by the budding are called the *secondary spores* or *sprout cells*.

The basidiospores or sporidia or secondary basidiospores are dispersed by wind and thus they may happen to fall on the soil or on the host plant. They bring about new infection of the host plants.

Agaricus: Life cycle

Introduction

The genus *Agaricus* comprises a group of 200 species which are heterotrophic, true nucleated, multicellular, macroscopic and fleshy in nature. This group includes fungi whose fruiting bodies are commonly known as mushrooms. The umbrella shaped mushrooms (which are edible), toadstools (which are nonedible or poisonous) beautify this earth with their presence in almost all seasons except the extreme summers and winters. The fruiting bodies of some species appear in early spring and disappear in summers, others appear only in rainy season and some appear sporadically whenever moisture is available. Hence the moist season (rainy weather) is the best season to explore, observe and collect the most beautiful, colourful (earthy flowers) fruiting bodies of mushrooms in hills as well as in plains.

The somatic structure

The somatic structure or vegetative thallus of *Agaricus* consists of the mycelium (mass of hyphae) which is septate, thin walled, hyaline typically a basidiomycetous type. Here it arises as a primary mycelium from the monokaryotic basidiospores, which later become dikaryotic and tertiary mycelium.

The mycelium of *Agaricus* is of three kinds:

1. **Primary mycelium:** The monokaryotic, haploid, basidiospore of (+) and (-) strain germinates immediately after dispersal on the substratum or on the moist soil (at 10–15 degree temperature, 80–90% relative humidity, and 5.8–6.0 ph) by absorbing mineral nutrients directly in soluble form to give rise a germling or primary hyphal initial. This hyphal initial cell undergoes mitotic division to form monokaryotic, uninucleated, haploid, hyaline, thinwalled primary mycelium which has a very short life in nature.
2. **Secondary mycelium:** The primary hyphae or monokaryotic hyphae with (+) or (-) strain haploid nuclei come in contact with each other to form dikaryotic or hetokaryotic mycelium by somatogamy. [Somatogamy: The somatic cells (vegetative cells) of primary or monokaryotic hyphae of opposite strains (+ and -) when they come in contact with each other directly on the substratum, the cell wall between the two cells (+ and -) dissolves, fusion of two protoplasts (plasmogamy) takes place, two nuclei (+ and -) lie near each other to form a dikaryotic cell (somatogamy).]
3. **Tertiary mycelium:** The dikaryotic, secondary mycelium carrying opposite strain (+and-) haploid nuclei in the hyphal cells branch profusely by mitosis and the hyphal branches anastomose with each other to form a complex tissue of fruiting body (basidiocarp).

Reproduction

Asexual reproduction

The species of *Agaricus* undergo asexual reproduction very rarely. It produces two types of asexual spores i.e. *oidium* (thin walled, fragmented spore), and *chlamydospores* (thick walled, resting spore).

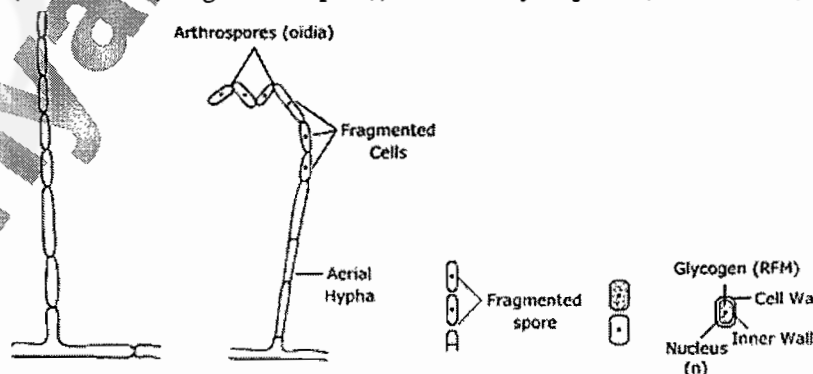


Figure 1: Asexual reproduction by oidia formation (rare)

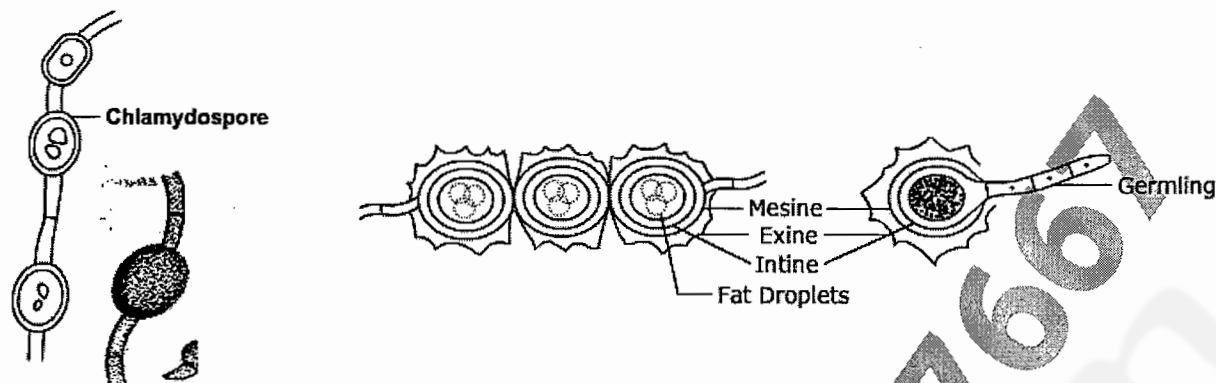


Figure 2: Chlamydospores and their germination

Sexual reproduction

Agaricus does not have well defined sex organs or sexual gametes. The somatic or vegetative cells of primary hyphae of opposite mating types (+&- strains) function as sexual gametes. The two somatic cells of haploid primary hyphae of opposite strains (+&-) come in contact with each other by a process of somatogamy.

After somatogamy, there is a prolonged dikaryotic phase.

The dikaryotic hypha proliferates by mechanism of clamp connection.

The fruiting body, the **basidiocarp** (commonly called **mushroom**) develops from the aggregation of dikaryotic hypha (Fig 3).

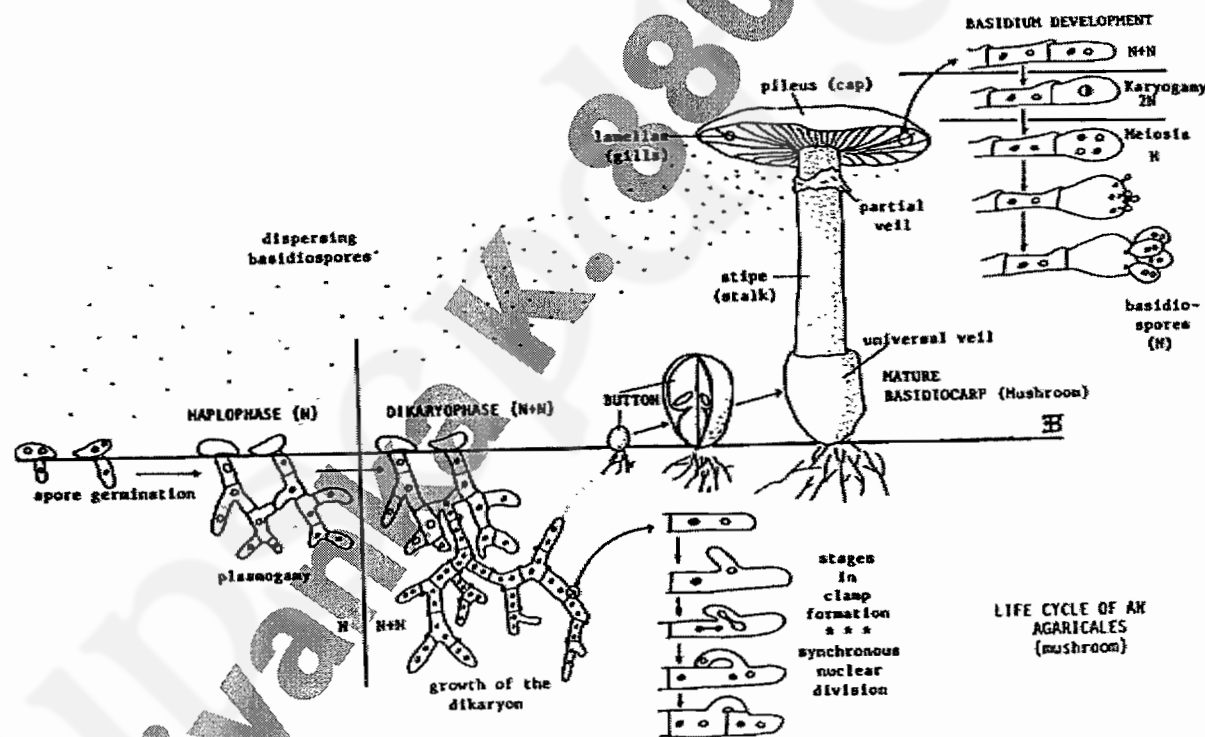


Figure 3: Development of basidiocarp in *Agaricus*

The fruiting body or the basidiocarp

The fruiting body or the basidiocarp formation is regulated or influenced by the interaction of multiple environmental factors:

- Light: Diffused light.
- Relative humidity: 80-90 %.
- Temperature: 12°-18° C.
- pH of the substratum: 5.8-6.0.
- Mineral nutrients (ammonium salts etc.) and aeration.

The initial steps in fruiting body formation are :

- An intricate hyphal lattice is formed by the interaction of hyphal branches .
- From this hyphal lattice the bunch of aerial hyphae appear which eventually produce a round aggregation of tightly interwoven hyphae to form a button or a bud like structure, bud primordium (first basidiocarp stage) on the substratum.
- The bud primordium grows in size and as it reaches 1.0 mm in diameter, a presumptive stipe, hymenium (fertile layer) and terminal pileus (cap) get differentiated simultaneously .
- The elongation of stipe, expansion of terminal pileus in to upper smooth and lower infolded gilled lamellar structure and production of basidiospores occur quite rapidly and dramatically in the life cycle of *Agaricus*.

The basidiocarp of *Agaricus* which bears basidiospores consists of the following tissues:

1. **Hymenium:** The fertile layer of anastomosed secondary hyphae is found beneath the pileus (terminal cap). It lines in the gills or lamellae exogenously which hang below in the ventral (abaxial) surface of the pileus.
2. **Gills:** The gills are the thin strips of tissues which radiate from the margin of the pileus in towards the stipe. The structure and position of the inner edge of gills towards stipe is a valuable and interesting feature in many agaricales for taxonomists. The gills in some species are free from stalk while in others they are attached directly to the stipe called adnate. The decurrent gills are attached and run down the stipe for some distance.
3. **Trama:** The trama is the inner most tissue which lie in the center of the gills.

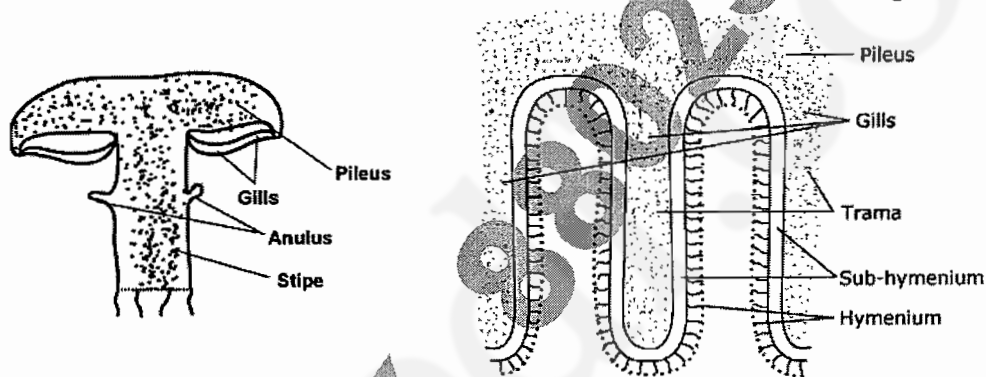


Figure 4: Diagrammatic representation of V.L.S. fruiting body and V.L.S. gills

The basidia are nothing but hyphal tips in the hymenium region. In these tips, first karyogamy occurs and then meiosis. This produces 4 basidiospores. (Fig. 5)

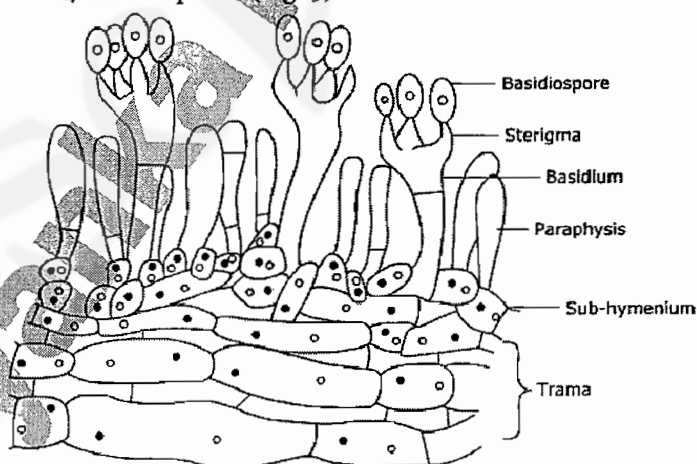


Figure 5: Part of gill magnified

Basidium: The basidium is a cell or a structure found at the terminal end of dikaryotic hypha in the hymenial layer of the gill exogenously. In the basidial cell the dikaryotic nuclei fuse with each other (karyogamy) to form diploid nucleus ($2n$) which immediately undergoes meiosis (reduction division) to form 4 basidiospores .

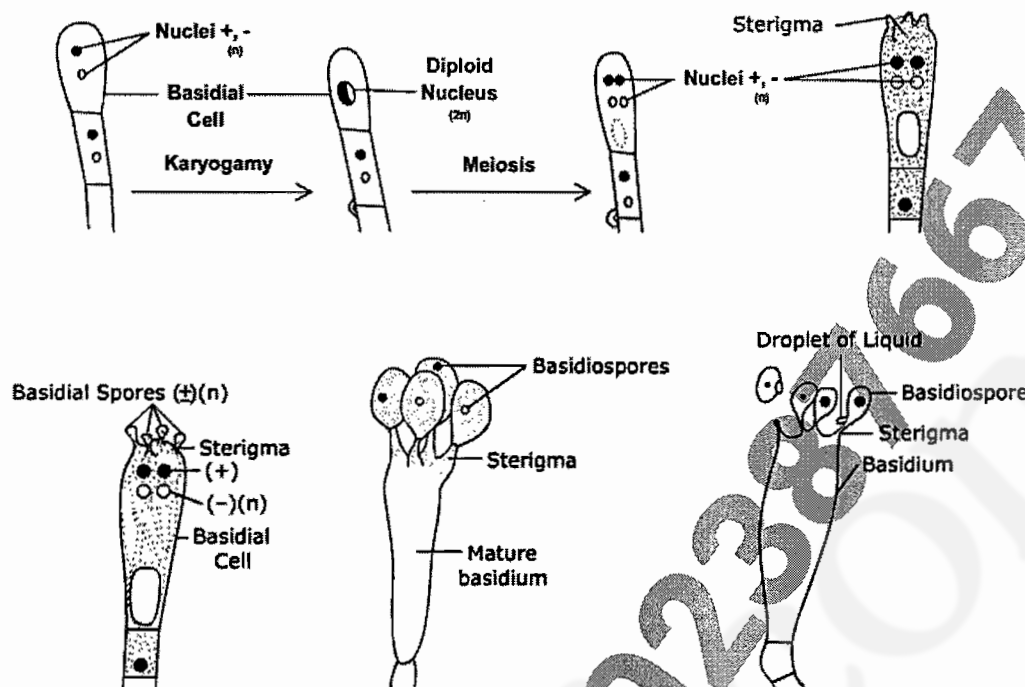


Figure 6: Development of basidium and formation of spores

Mushroom cultivation in India

Introduction

Mushrooms are fungi, fleshy in nature, rich in proteins, minerals and vitamins and can form a rich vegetarian diet full of taste, unique flavour, aroma and nutrition. Mushrooms found in nature are either edible or poisonous and out of 1600 mushrooms identified worldwide, nearly 100 are used as food and 33 types are under commercial cultivation.

In India the mushrooms under cultivation are *Agaricus bisporus* (white button), *Pleurotus* (Oyster) and *Volvariella* spp. (paddy straw) and the sporadic cultivation of *Auricularia* spp. (black ear) and *Calocybe indica* (milky mushroom) has begun in recent years.

The commercial cultivation in India have begun in 1971, the annual production of which was 100 tons and now it is more than 60,000 tons.

Places of cultivation

In India the mushrooms are cultivated in large scale in Punjab, Haryana (Sonapat), Himachal Pradesh, (Solan), South India etc.

The Mushroom Research Laboratories at Solan have excelled in standardizing the techniques for raising *Agaricus* (white button) and Oyster and also helped in establishing mushroom growing centers in Chail, Shimla, Kasauli and 116 other mushroom farms all over the country by supplying mushroom spawns to the growers.

Economic Importance

Mushrooms decompose the agro waste (cellulose, hemicelluloses, and lignin) and organic waste materials and utilize them to produce edible biomass of high nutritive value. Mushrooms are considered vegetarian and are rich in proteins, vitamins, and minerals which can form a purely vegetarian diet with good taste and aroma.

Food value

Cultivated mushrooms are low in calories (135 in 1 kg) rich in proteins, vitamins and minerals. The mushroom fruiting body on dry weight basis contains:

- Carbohydrates-55%
- Proteins -32%
- Fat - 2%

- Minerals - macro elements - P, K, Cu, Fe - micro elements - Na, Ca, and Mg
- Vitamins - thiamine (B1), riboflavin (B2), niacin, pantothenic acid (B complex), biotin, folic acid, vitamin C, D, A and K(these are retained even after cooking).

Mushroom proteins contain all the nine essential amino acids basically needed for human growth.

It is easily digestible (71 -90%) and 200 gm of mushroom protein is comparable to 100 gm of non vegetarian protein (meat).

It is an alternative source of protein and can be used for poor people in the developing countries.

Mushroom Cultivation

In India *Agaricus bisporus* (white button) is cultivated in large scale and it contributes nearly 90 - 95% in the Indian market and the rest comes from *Pleurotus* spp. (oyster) and *Volvariella* spp. (paddy straw).

Growing season

Mushrooms are cultivated through out the year in northern Indian plains and in a particular season:

- October to March: *Agaricus bisporus* (White button mushroom).
- May to July: *Volvariella* spp (Paddy straw mushroom).
- Mid August to mid April: *Pleurotus* spp.(Oyster mushroom).
- February to April: *Calocybe indica* (Milky mushroom).

Specific climatic conditions

The mushrooms require specific climatic conditions: The R.H. 85-90%, PH 5.8-6.8 and the temperature requirement for the spawn run (mycelium) and fruiting (reproductive bodies) of mushrooms under cultivation are:

Mushroom spp.

Temperature °C

Mycelium Growth

Best Fruiting

Agaricus bisporus

22 - 25

14 - 18

A. bitorquis

24 -30

17 -24

Auricularia spp.

20 -34

12 - 30

Lentinus edodes

20 -27

10 -20

Pleurotus eryngii

18 - 22

14 - 18

P.Flabellatus

25 - 32

22 - 26

Volvariella volvacea

20 - 25

28 - 32

Calocybe indica

25 - 35

26 - 30

Cultivation of (White button) *Agaricus bisporus* mushroom

Agaricus bisporus (=A. *brunneescence* ; approximately annual yield 2,000,000 t.) is the most favourite amongst other mushrooms under cultivation grown commercially in the world. In India it is grown mostly in large scale in the south, west and northern regions through out the year (to meet the export as well as home demands), in the mushroom houses under controlled conditions to procure nearly 5 crops annually. The seasonal cropping is done from October to mid March to collect one crop, but with specific precautions two crops of 6-7 weeks duration can be taken.

The *Agaricus* being heterotrophic in nature does not need fields (large areas) and direct sunlight for its cultivation like other conventional angiospermous crops. It is cultivated in the large scale in mushroom houses on the substratum like wheat or rice straw supplemented with horse dung, poultry manure, sugarcane bagasse, wheat or rice bran, molasses and chemical fertilizers. All these materials are mixed thoroughly, fermented aerobically and this process is called composting. During this process the nutrients of the raw material used for making the compost get ready to be used to grow and develop the mushroom mycelium and resist the growth of other micro -organisms.

The process of cultivation under controlled conditions and the essential apparatus required facilitating the composting and cropping of mushrooms is:

- Mushroom house
- Composting yard
- Ingredients and raw material for natural and synthetic compost
- Spawn / seed of mushroom.
- Casing
- Equipments, accessories and pesticides.

Mushroom house

The button mushroom can be grown indoors in rooms, sheds, garages, basements etc with good ventilation. A good mushroom house should be equipped with the following apparatus:

- It should be in an area free from air pollution.
- It should be well ventilated and made up of insulated walls.
- The ventilators to be fitted with 30 mesh size net.
- The light arrangement of 1500 – 2500 lux intensity.
- The exhaust fan, cooler and air- conditioner.
- It should have pucca floor of either cement or bricks.

The racks to be made at least 15 cm apart from the wall and floor of the mushroom house. At the opening of the door of mushroom house a 4-5 cm deep pit of door mat size to be made and to be filled with 4% formalin solution and a jute bag to be put in place of doormat to check pollution from outside during cropping period.

Composting yard

Compost is prepared over composting yard i.e. an area where the raw materials like agrowaste, chemicals etc are mixed and fermented. It should have the following:

- It has to be at the corner of the mushroom farm and away from mushroom house.
- A pucca floor of cement or brick.
- A tin shed over the composting yard to avoid rainy water.
- The floor with a slope with the provision of drainage in a pit to reuse that water for wetting of the compost.

Natural and synthetic compost ingredients and raw materials

Natural compost: The natural compost is prepared by fresh horse dung collected from stables mixed with 1/3 weight of wheat or barley straw, 100-110kg chicken manure and 3kg urea per tonn and made into a heap of 1meter high. This mixture must be kept under shed and be protected from rain. The horse dung should not contain the admixture of dung from other animals. The heap after 3-4 days begins to steam due to fermentation, rise in temperature and the production of ammonia. The heap is opened and this process is repeated 4 or 5 times at 5 or 6 days interval. Gypsum 25kg/ton of horse dung is added at 2 nd and 3rd turning and 40ml nemagon is sprayed at the final turning into the manure. Now the compost is ready to use in mushroom cultivation.

Synthetic compost: This compost is prepared by either long method (traditional) or by short method (pasteurized). The raw materials used for composting (formulae) have been given by different organizations, institutions and workers and can be selected for making compost. Here a formula is given to be used in synthetic compost:

Formula - 1

Material	Quantity
Chopped wheat straw/ paddy straw	250 kg (10 - 15 cm SIZE)
Wheat bran	25 - 30 kg
Ammonium sulphate /CAN (calcium ammonium nitrate)	4 kg
Urea	3 kg
Gypsum	20 kg
Malathion	15 - 20 ml

Compost preparation

Compost is prepared by two methods i.e. Long method and Short method.

Long method:

1. Wheat straw is spread in a thick layer of 8-10" thickness over the floor of composting yard.
2. Sprinkle water on the straw for wetting 2-3 times a day for two days.
3. Urea, CAN, and wheat bran are thoroughly mixed separately and covered with damp gunny bag for 14-16 hours.
4. These ingredients are now mixed with a pre wetted straw on the floor and is heaped into a pile with a stack mould.
5. The entire pile is opened and spread over the composting yard on 3rd or 4th day for 45-60 minutes and this process is called turning which is repeated on every 3rd day. At each turning water is sprinkled to make up the loss of water due to evaporation. At 3rd turning 1/2 of gypsum amount is

added and the remaining gypsum is added on 4th turning. At 5th turning insecticide nemagon is added and thorough mixing of straw is done, later open the pile and leave it for 3 days till ammonia smell is lost.

6. The compost is ready to use in 18-21 days, in which the ligno-protein complex is formed that favours the growth of white button mushroom, also narrows down carbon /nitrogen ratio with the addition of nitrogen sources.

The Short or Pasteurization Method: Here, the compost is formed in two stages:

Stage 1:

The wheat straw is moistened, mixing of ingredients, making a heap and turning is given in 2 days, gypsum is added after 3rd turning, and after 4th turning the compost is filled in pasteurization tank.

Stage 2:

The temperature 48-50°C is maintained in pasteurization tank for 2-3 days. Steam is passed to raise the temperature to 58-60°C for 6 hours. Fresh air is allowed through ventilation till the temperature of the compost cools down to 25-28°C, and the compost is ready in 19-20 days.

Spawn/seed of Mushroom

Mushroom seed is called spawn, that is raised from the healthy strains, thoroughly tested and recommended for a particular area or zone. It is the creamish white thread like mycelium of the mushroom growing on the medium (wheat or other cereal grain) that provides it nutrition for its growth. Spawn that is used as seed must be free from contaminants (micro-organisms), fresh or only one month old and can be stored at 5°C. The spawn of 160-170 gm is used in one square meter area of the compost of 15-16 cm thickness. The quantity of spawn used at 22-26°C on dry straw is 1-1.5% and 0.50 -0.75% on wet compost. In hills or during winters in plains spawn is required in more quantity i.e. 2.5-3.0% of the dry weight of the straw.

Casing

Casing material is a soil which is, powdered, thoroughly sieved, porous with neutral pH, which allows free exchange of air, also can retain water and is used to cover the compost after spawn run. It is a process of covering the spawn run compost with casing soil to initiate fruiting (due to shock treatment), to support the fruiting body and to check the loss of moisture from mushroom compost.

Equipment, accessories and pesticides

Mushroom trays, foot sprayers, sharp knife, gunny bags, water storage drums, plastic/alkathene sheet, water pipe and insecticides etc. These equipments are used in mushroom house in various ways.

Process of Cultivation

The seasonal sowing of white button mushroom (*Agaricus bisporus*) is done during October to mid of March in Delhi, Haryana, Punjab, Rajasthan and Uttar Pradesh. The recommended strains of *Agaricus bisporus* are P-1, S-11, Pant -52, NCS-5, NCS-14, S-310, MS-39 etc.

Compost preparation is completed in the last 20 -25 days of September and Wheat and paddy straw are best substrates for *Agaricus* cultivation. The compost ready for spawning is dark brown in colour, without any odour of ammonia and has sufficient moisture when pressed between the palms.

Filling of the compost

The prepared compost is filled in trays and racks of 6-8" thick layer, lightly compressed and levelled.

Spawning

Sowing the beds with the mycelium (spawn) is called spawning.

After spawning the trays are covered with old newspaper sheets and watered lightly with sprayer to provide moisture. The trays are then stacked vertically one over the other in 3-4 tiers. In bag cultivation, bag mouth is tied with thread or sutli.

Casing

The casing is done after the completion of spawn run and removal of news paper from trays or racks and opening of mouth of polybags with 4-5 cm thick layer of casing soil. After casing water is sprayed over casing soil and the temperature and relative humidity is maintained.

Fruiting/crop

The induction of bud primordia or pin heads or fruiting bodies on the casing soil is observed after 15-20 days of casing. The casing layer is frequently wetted with mist spraying of water. At this stage large amount of fresh air is required.

Harvesting

The harvesting begins when the cap size is 3–4cm in diameter. The right stage of harvest is when the cap is still tight and membrane below the cap is intact over the short stipe. In case the buttons are allowed to mature further the cap will rupture, dark coloured spores will be exposed and mushrooms become inferior and loose market value. Pre-harvesting spray of 2% ascorbic acid improves the colour (whiteness) by inhibiting the polyphenol oxidase enzyme activity.

Picking is done by holding the cap with fore fingers slightly pressed against the soil and rotating it in anticlock-wise. The soil particles and mycelial threads clinging to the base of the stipe is removed with knife. Harvesting is also done with knife by cutting the stipe at the soil level.

Oomycota

Oomycetous Fungi

The term Oomycetes describes a class of organisms which were once considered Fungi but now regarded as Fungi Like Organisms (FLOs) rather than 'true' Fungi. In the traditional classification systems of the fungi, such as the one given by G.C. Ainsworth in 1971, the Oomycetes were considered a class of Mastigomycotina (the fungal subdivision whose members produce flagellated cells at some stage of the life cycle). However, modern phylogenetic understanding of the fungi has led to their exclusion from the Kingdom Mycota and placement in the Kingdom Stramenopila (Patterson and Sogin, 1992).

The important features of the oomycetous organisms are as follows:

1. They are filamentous and microscopic.
2. The hypha is aseptate and multinucleate in the trophic stage.
3. The hypha lacks chitin in most of the genera but contains glucans as microfibrillar component.
4. The mode of nutrition is absorptive.
5. The reserve food is Mycolaminarin and not glycogen like most fungi.
6. They reproduce both sexually and asexually.
7. Asexual reproduction is based on Zoospores. The zoospores are mostly reniform (kidney shaped).
8. There are two laterally inserted flagella in the zoospores. Both the flagella are unequal in length. The longer flagellum is tinsel type and the shorter one is whiplash type. The tinsel flagellum is pointed anteriorly and the whiplash flagellum is pointed posteriorly.
9. Sexual reproduction involves oogamous mode.
10. All members have large round oogonia, structures containing the female gametes. This feature is the characteristic of the oomycetes. Each oogonium contains 4, 8, or 16 eggs. The sexual process results into Oospore.
11. The life cycle is diploid dominant type.

The oomycetes are unique from the rest fungi in several ways as summarized in the table below.

Table 1. Major distinctions between the Oomycota in the Chromista and the true Fungi (Chytridiomycota, Zygomycota, Ascomycota, Basidiomycota)

Character	Oomycota	True Fungi
Sexual reproduction	Heterogametangia. Fertilization of oospheres by nuclei from antheridia forming oospores.	Oospores not produced; sexual reproduction results in zygosporangia, ascospores or basidiospores
Nuclear state of vegetative mycelium	Diploid	Haploid or dikaryotic
Cell wall composition	Beta glucans, cellulose	Chitin. Cellulose rarely present
Type of flagella on zoospores, if produced	Heterokont, of two types, one whiplash, directed posteriorly, the other fibrous, ciliated, directed anteriorly	If flagellum produced, usually of only one type: posterior, whiplash
Mitochondria	With tubular cristae	With flattened cristae
Reserve food	Mycolaminarin	Glycogen
Hyphal structure	Coenocytic and aseptate	Septate in Ascomycota and Basidiomycota.

In the recent years, the ultrastructure, biochemistry, and molecular sequences of these organisms indicate that they belong with the Kingdom Stramenopila (Alexopoulos *et al.* 1997). Some authors place the oomycetes in the Kingdom Chromista, phylum Heterokonta. The Kingdom Chromista includes several kinds of algae, namely the Phaeophyta or brown algae, Xanthophyta or yellow-green algae, Chrysophyta or golden algae, and Bacillariophyta or diatoms. The free-swimming spores with two dissimilar flagella, one

with mastigonemes and the presence of the chemical mycolaminarin are features similar between the Oomycetes and algal members of chromista.

Thus, the current position of the Oomycetes remains controversial, though it is well established that they are not fungi.

Life cycle of *Phytophthora*

Phytophthora is a genus of order Peronosporales in the Phylum Oomycota within kingdom Stramenopila. The scientific name *Phytophthora* means "plant destroyer" in Greek. The genus includes many plant pathogens of dicotyledons with considerable economic importance. Important pathogenic species include:

1. *Phytophthora infestans* (Potato blight — the disease behind the Great Irish Famine between 1845 and 1851, that killed more than 500,000 people.)
2. *Phytophthora cactorum* (Collar rot)
3. *Phytophthora cinnamomi* (dieback) - this affects as many as 2000 of the 9000 native plant species in Southwest Australia, most notably *Eucalyptus marginata*
4. *Phytophthora sojae* (Soybean root rot) - a major cause of crop loss.

Structure

The organization of somatic body is mycelial, with the hyphae being the basic structural units. The hypha is hyaline, branched, aseptate and coenocytic. It grows apically – like any other hypha. The cell wall of hyphae mainly consists of glucan and not chitin. This is a common feature of Oomycota and an important reason why these organisms are no longer considered as true fungi, but actually fungus like organisms. The hyphae produce haustoria for the absorption of food material from the host cell.

Life cycle of *Phytophthora infestans*

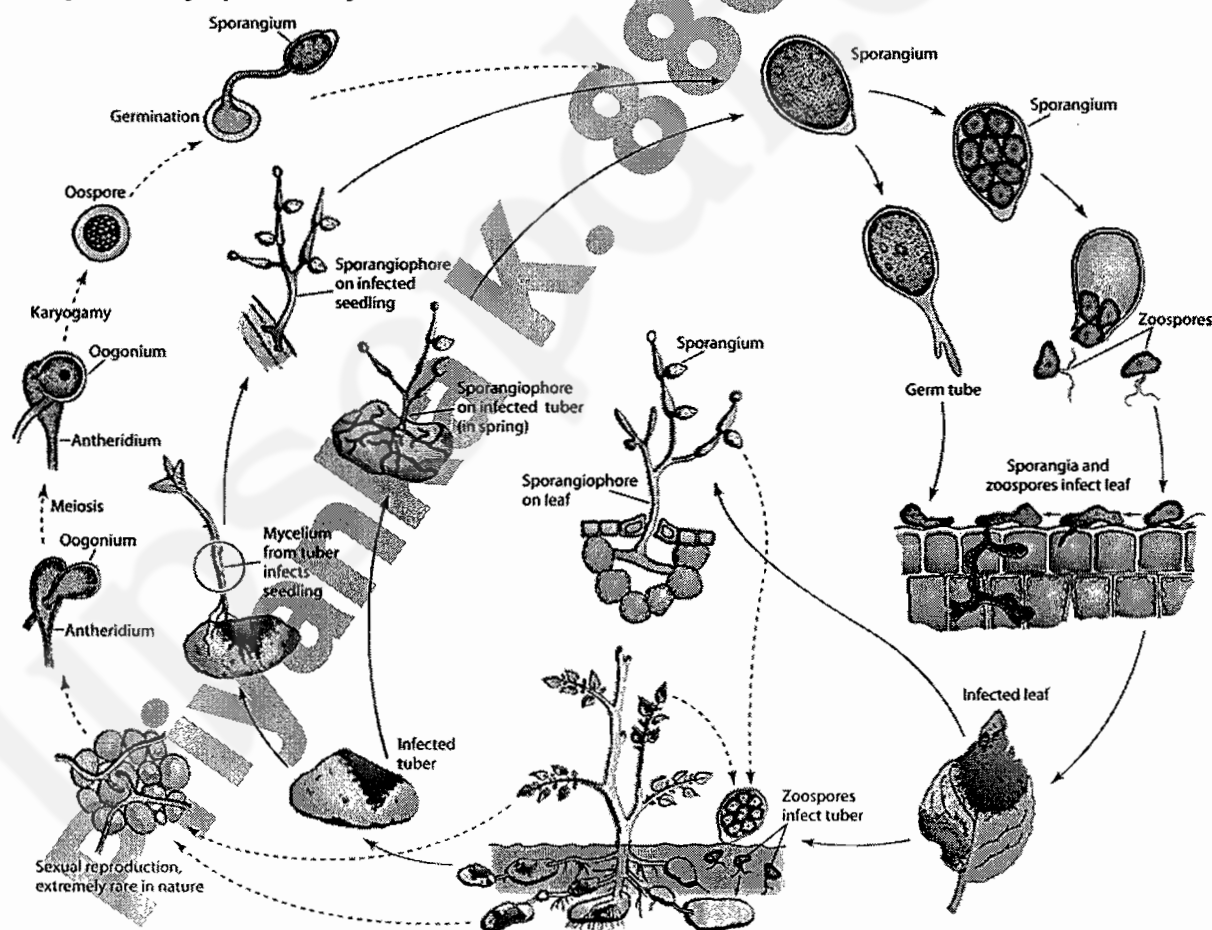


Figure 1: Life cycle of *Phytophthora infestans*

Phytophthora reproduce both sexually and asexually.

Asexual reproduction

- Asexual reproduction takes place by zoospores formed in detachable sporangium. Some authors have called this structure as Conidiosporangium.
- Under favourable conditions, i.e. at temperature of 20°–22°C and 100% atmospheric relative humidity, the hyphal branches from the intercellular mycelium emerge out through stomata or by piercing epidermal cells. These hyphal branches act as conidiophores or sporangiophores.
- A sporangium develops at the tip of the sporangiophore. The first sporangium on a sporangiophore is terminal, but soon it comes to lie in a lateral position as the sporangiophore pushes the previously formed sporangium to one side to produce another sporangium.
- The sporangium is pear-shaped or oval and thin walled with a terminal papilla or beak like projection. The mature sporangia are detached from the sporangiophore and are dispersed by wind, rain splashes or by contact with other leaves.
- On a suitable substratum the sporangia germinate — directly or indirectly.
 - In indirect germination (occurring under high moisture conditions), each sporangium acts as zoosporangium which numerous zoospores are formed. In this process, the multinucleate protoplast of the sporangium divides into 5–10 uninucleate segments. Each segment then metamorphoses into a biflagellate reniform zoospore. The flagella of the zoospore are lateral; one of them is of whiplash type and the other of tinsel type. After liberation, the zoospores swim in a thin film of water for some time, then withdraw their flagella and secrete a thick wall. These cysts germinate in favourable conditions by producing germ tube.
 - In direct germination (occurring under low moisture conditions), the sporangium acts as a conidium. The conidium produces a germ tube which ultimately grows into a new mycelium. This germ tube is enclosed within a thin wall called germination wall.
- The germ tube whether arising directly or indirectly, ultimately penetrates the host tissue by an appressorial mechanism — leading to an infection.

Sexual reproduction

Sexual reproduction is **heterogamous** and occurs by direct injection of the male nuclei (= sperms) from the **antheridium** into the eggs contained in the **oogonium**. A swimming sperm is absent in the Oomycota. This type of sexual reproduction is referred to as **gametangial contact**. The eggs and sperms are products of meiosis and the only parts of the life cycle that are haploid. Otherwise, the members of Oomycota have a **diploid dominant lifecycle**.

On the basis of the development of gametangia, species of *Phytophthora* may be classified as paragynous or amphigynous.

- In paragynous species (e.g., *P. ractorum*), first an oogonium is formed and then from the same or nearby hypha an antheridium develops. In such species, therefore, antheridia are attached laterally to the oogonium.
- In amphigynous species (e.g., *P. erythrosetica*; *P. himodaysensis*, *P. infestans*) first an antheridium is formed. When the antheridium is still in the developing stage, a nearby hypha emerges out by piercing the antheridium. The tip of this hypha inflates to form a spherical oogonium. Thus, in these species the antheridium appears as a collar above the base of the oogonium.

Pythium life cycle

A basic introduction to the genus

- *Pythium* is a genus of order Peronosporales in the Phylum Oomycota within kingdom Stramenopila. (Formerly treated as Oomycetes within Mastigomycotina by GC Ainsworth's system and other classical systems of fungal systematics).
- *Pythium* is the causal agent of root rot, which is a common crop disease.
- *Pythium damping off* is a very common problem in fields and greenhouses where the organism kills newly emerged seedlings.
- Many *Pythium* spp., along with their close relatives, *Phytophthora* spp. are plant pathogens of economic importance in agriculture.
- *Pythium* spp. are very generalistic and unspecific in their host range — that is, they infect a large range of hosts (Owen-Going, 2002), while *Phytophthora* spp. are very specific, i.e. *Phytophthora capsici* only

infect pepper (*Capsicum* spp.) and *Phytophthora infestans* only infect potatoes. For this reason, *Pythium* spp. are more devastating in the root rot they cause in field crops, because crop rotation alone will often not eradicate the pathogen (nor will following the field, as *Pythium* spp. are also good saprotrophs, and will survive for a long time on decaying plant matter).

- However, the damage *Pythium* spp. does in field crops is limited to the area affected, because the motile zoospores need ample surface water to travel long distances as the capillaries formed by soil particles act as a natural filter.
- In hydroponic systems inside greenhouses, where extensive monocultures of plants are maintained in plant nutrient solution (containing nitrogen, potassium, phosphate and micronutrients) that is continuously recirculated to the crop, *Pythium* spp. cause extensive and devastating root rot.

Somatic Structure

The organization of somatic body is mycelial, with the hyphae being the basic structural units. The hypha is hyaline, branched, aseptate and coenocytic. It grows apically — like any other hypha. The hyphae are 4-8 mm in diameter and their wall is about 0.1 mm thick. The cell wall of hyphae mainly consists of glucan and not chitin. This is a common feature of Oomycota and an important reason why these organisms are no longer considered as true fungi, but actually fungus like organisms.

The hyphae produce haustoria for the absorption of food material from the host cell.

Reproduction

Pythium reproduces both sexually and asexually.

Asexual reproduction

It takes place by zoospores which are formed in the sporangia. The sporangia are either terminal on intercalary on the vegetative hyphae.

Each sporangium has an apical papilla, through which sporangial contents are discharged into a vesicle. In the vesicle are formed many biflagellate zoospores. The zoospores are kidney - shaped and have two lateral flagella attached on the concave side. They are liberated by the bursting of the vesicular wall. After a period of swarming the spore comes to rest, encysts, and germinates by a germ tube.

Sexual reproduction

The sexual reproduction is homothallic or heterothallic oogamous. Plasmogamy that precedes sexual karyogamy is achieved by Gametangial Contact.

Oogonia and antheridia are developed in close proximity, usually with the antheridium just below the oogonium. The pattern of growth of the sex organs follows the standard pattern of the Oomycetes.

Fertilization: At maturity, the antheridium gives out a fertilization tube through which the male nucleus passes into the oogonium and unites with its nucleus thus affecting fertilization. The resulting oospore develops a thick and ornamented wall.

The oospores undergo a period of rest of several weeks before they germinate. When the temperature is very high, it germinates by germ tube that develops into a mycelium. However, at lower temperature, the germ tube stops growing and forms a vesicle at its tip. Inside the vesicle, zoospores develop which on liberation develop into a new mycelium.

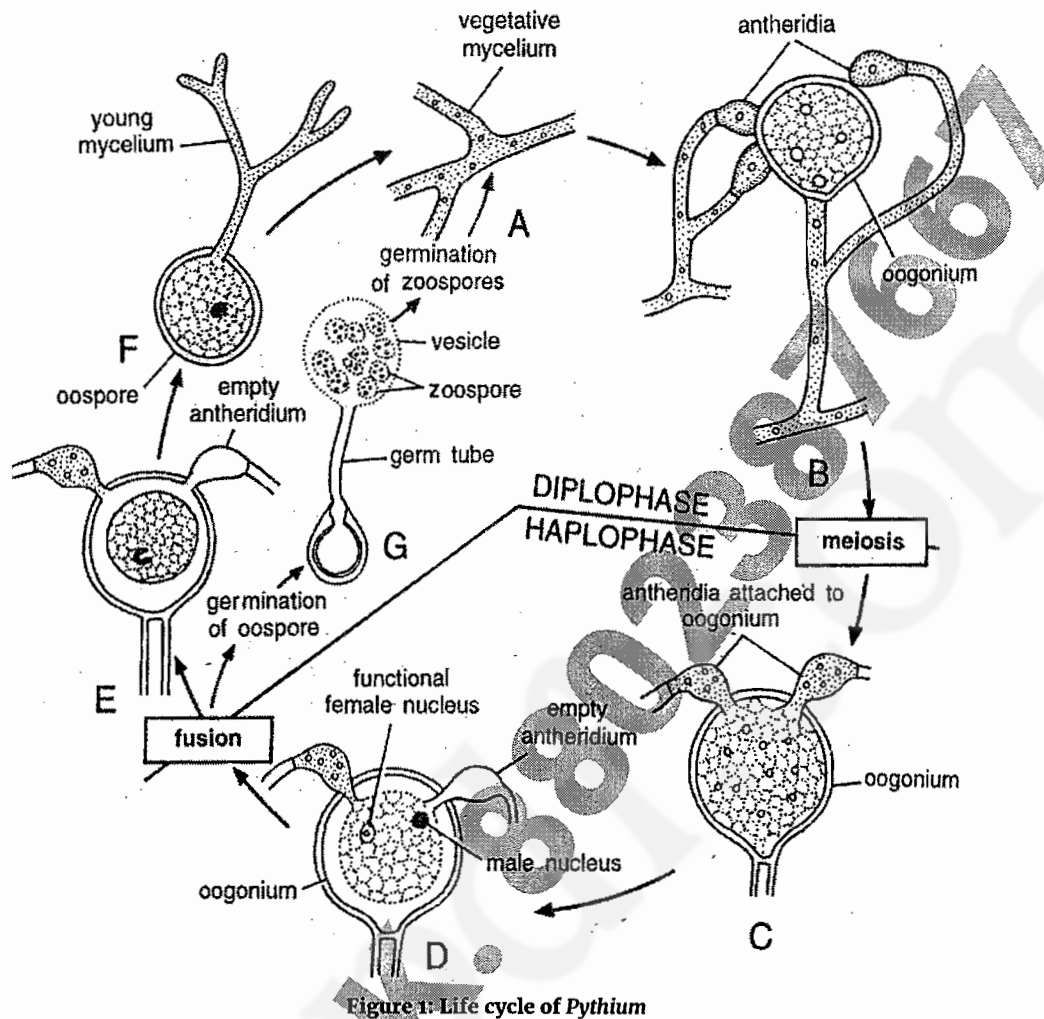


Figure 1: Life cycle of *Pythium*

Life cycle of *Albugo*

Albugo is a genus of order Peronosporales in the Phylum Oomycota within kingdom Stramenopila. (Formerly treated as Oomycetes within Mastigomycotina). The older name scientific name for the imperfect stage of the genus was *Cystopus*.

The genus *Albugo* is represented by about 30 species, distributed in the most parts of the world.

They are **obligate parasites** occurring as endoparasites in higher plants. *Albugo* spp. affect only or primarily the aboveground tissues of their hosts, in particular the leaves, young stems, and fruits.

They usually infect the members of the families Cruciferae, Convolvulaceae, Compositae, Amaranthaceae, etc. *A. candida* (= *Cystopus candidus*) is the most common species of the genus and it parasitizes the members of Cruciferae, like radish, turnip, cabbage and mustard and **causes white rust** or blisters of crucifers.

Structure

The mycelium of *Albugo* is well-branched, aseptate and coenocytic. The fungus grows in intercellular spaces of the host tissue and absorbs food material from the host cell with the help of small knob-like haustoria. The haustorium is differentiated into a haustorial head (about 4 µm in diameter) and a slender neck-like stalk (about 0.5 µm in width). In the haustorial head, the cytoplasm is dense and packed with mitochondria, ribosomes, endoplasmic reticulum and lipid inclusions, but nuclei are, however, absent. The base of the haustorium is surrounded by a collar-like sheath which is an extension of the host cell wall. The haustorium is separated from the host plasma membrane by an encapsulation made up of extra-haustorial matrix.

Reproduction

Albugo reproduces both by asexual and sexual means.

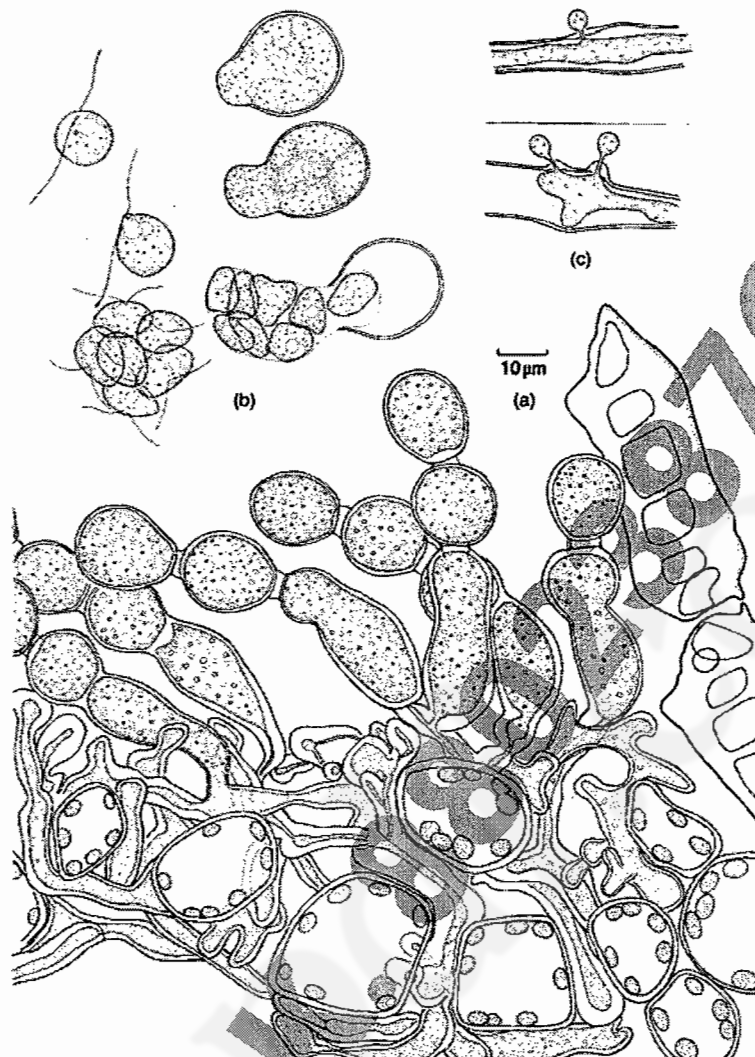


Figure 1: *Albugo candida* on *Capsella bursa-pastoris*. (a) Mycelium, sporangiophores and chains of sporangia formed beneath the ruptured epidermis (right). (b) Germination of sporangia showing the release of eight biflagellate zoospores. (c) Haustoria.

Asexual reproduction

The intercellular mycelium collects beneath the host epidermis. The tips of these hyphae develop into short, erect, thick-walled and club-shaped structures, called conidiophores or sporangiophores.

Asexual reproduction takes place by multinucleate structures, known as conidia, sporangia or zoosporangia. These structures develop in long basipetal chains on sporangiophores. These structures are formed after the pathogen has established in the host tissue.

They develop as a palisade-like layer beneath the host epidermis and lie perpendicular to the host surface. The apical end of the canidiophore is multinucleate, thin walled and densely cytoplasmic.

Sporangia produced in pustules, once liberated, are dispersed by wind, rain, or insects to neighboring plants. The sporangia require some drying in order to germinate well. Each germinating sporangium gives rise to five to seven zoospores. The preferred temperature for germination ranges from 1° to 18° C, but is optimum between 10° and 14° C.

Temperatures should be between 16° and 25° C, with the optimum at 20° C for zoospores to produce germ tubes and penetrate plant tissue. The moisture necessary for zoospore activity is ideal when in the form of heavy dew or fog or during periods of extended rainfall and lower temperatures.

If conditions are not favourable, conidia (sporangia) of some species of *Albugo*, such as *A. bliti*, *A. portulacae* and *A. spinulosa* do not form zoospores and instead they germinate directly.

The germ tube whether arising directly or indirectly, ultimately penetrates the host tissue by an appressorial mechanism — leading to an infection.

Sexual Reproduction

In *Albugo*, sexual reproduction is oogamous and the male and female sex organs are known as antheridium and oogonium respectively. In *A. candida* the sex organs are generally formed towards the end of the growing season of the host. They develop in intercellular spaces, quite deep into the host tissues.

Fertilization is heterogamous and occurs by direct injection of the male nuclei (= sperms) from the antheridium into the eggs contained in the oogonium. A swimming sperm is absent in the Oomycota. This type of sexual reproduction is referred to as gametangial contact. The eggs and sperms are products of meiosis and the only parts of the life cycle that are haploid. Otherwise, the members of Oomycota have a diploid dominant lifecycle.

There is some evidence of heterothallism in *A. candida*. One study examined the pathogen on a host species and noticed that primary infections were sterile but became fertile when secondary infections were established.

Plasmodiophoromycota

Introduction

The Plasmodiophoromycota are a group of obligate (i.e. biotrophic) parasites. The best-known examples attack higher plants, causing economically significant diseases such as club- root of brassicas (*Plasmodiophora brassicae*), powdery scab of potato (*Spongospora subterranea*) and crook-root disease of watercress (*S. nasturtii*). In addition to damaging crops directly, some species also act as vectors for important plant viruses.

Other species infect roots and shoots of non-cultivated plants, especially aquatic plants. Algae, diatoms and Oomycota are also attacked.

Plasmodiophoromycota are **fungi like organisms** and have been included in the protists (Patterson and Sogin, 1992; Mims, Alexopoulos and Blackwell, 1998, 2002).

They may be considered a monophyletic taxon because all members share certain derived characters, most importantly cruciform nuclear division. Other features of plasmodiophorids include a) zoospores with two, anterior whiplash flagella; b) multinucleated protoplasts (plasmodia); c) obligate, intracellular parasitism; and d) environmentally-resistant resting spores (cysts).

Plasmodiophora

Plasmodiophora is member genus of the Phylum Plasmodiophoromycota within kingdom Protista. (Formerly treated as Plasmodiophoromycetes within Myxomycota by GC Ainsworth's system and other classical systems of fungal systematics).

Plasmodiophora brassicae is the casual agent of club root disease of crucifers (Fig. 1). The disease was first reported in the United States of America in 1852. Historical reports of club root date back to the 13th century in Europe. In the late 19th century, a severe epidemic of club root destroyed large proportions of the cabbage crop in St. Petersburg, Russia.

The Russian scientist Mikhail Woronin eventually identified the cause of clubroot as a "plasmodiophorous organism" in 1875, and gave it the name *Plasmodiophora brassicae*.

Pathogenesis

Plasmodiophora brassicae can be found worldwide in all temperate zones. It infects over 300 species in 64 genera of crucifers and can be found in both cultivated and wild crucifers. Economically important hosts include cabbage, collards, kale, mustard, brussel sprouts, radish, turnip, rutabaga, cauliflower, broccoli, rape, and kohlrabi.

The most characteristic symptom of *P. brassicae* within a host, is the clubbing of host roots. Symptoms vary slightly from host to host.

The first observable above ground symptom is day wilting. As the disease progresses, leaves yellow and die. Diseased plants are obviously stunted compared to uninfected plants and will often be localized in low, wet areas of the field. When dug up, roots exhibit a variety of symptoms. New infections cause small knot like galls on roots, whereas more developed infections display long spindle shaped clubs on primary and lateral roots. Some hosts, such as turnips and radishes, do not form clubs when infected. These hosts have black sunken lesions along the root surface.

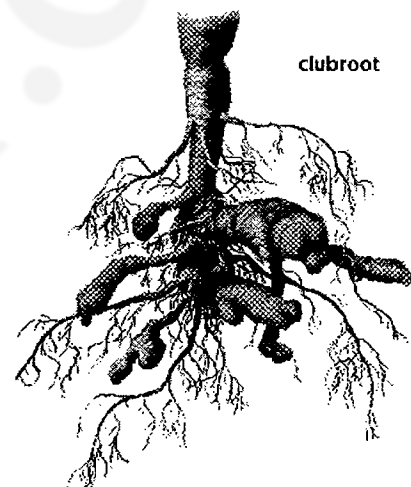


Figure 1: Club Root Condition

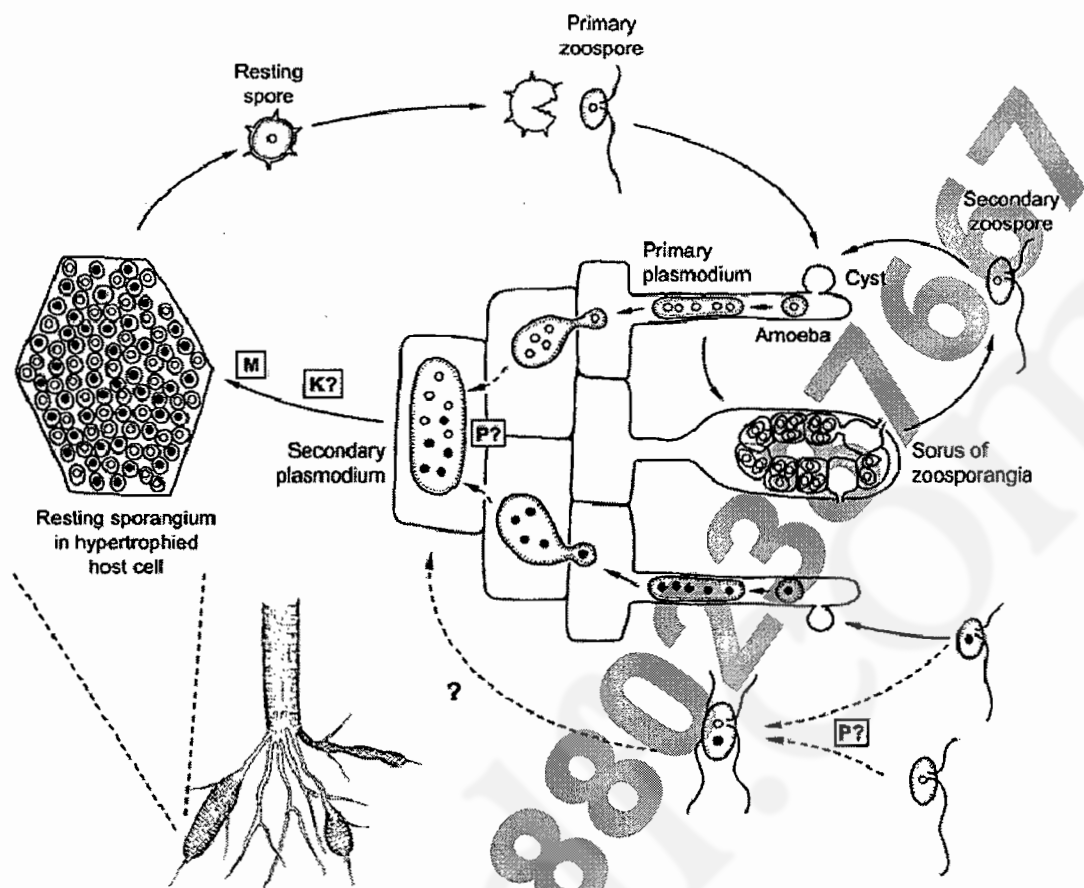


Figure 2: Probable life cycle of *Plasmodiophora brassicae*. A haploid resting spore forms a haploid primary zoospore giving rise to a multinucleate haploid primary plasmodium upon infection of a root hair. Secondary zoospores are also haploid, and the way in which they meet to form a secondary heterokaryotic plasmodium is not known for sure. Open and closed circles represent haploid nuclei of opposite mating type; the position of the diploid phase in the life cycle is unclear. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M).

Life Cycle of *Plasmodiophora brassicae*

- *Plasmodiophora brassicae* is an obligate parasite. It survives in the soil only as dormant cysts. Cysts can survive for up to 6–8 years without the presence of a host, and will germinate in response to the presence of crucifer root exudates.
- Primary zoospores are released from germinating cysts.
- They infect host root hairs by encysting on the root surface by a unique injection mechanism and entering through developing epidermal cells in the form of an amoeba like cell.
- Older roots can also be infected if wounding is present to provide an entrance to the pathogen.
- In the root hairs, amoeboid cells of the pathogen join together to form a multinucleate plasmodium. (Kubeta et al 2004)
- After a few days, this plasmodium cleaves into multinucleate portions, each of which develops into a zoosporangium. The zoosporangia are discharged from the host, where each releases four to eight zoospores (sometimes referred to as secondary zoospores).
- Secondary zoospores infect healthy parts of the initial host or infect nearby plants.
- These zoospores also enter through the host root hairs, but the infecting amoeboid cells migrate into the cortical cells of the host.
- Once in the cortex, the amoeboid pathogen infects one host cortical cell where it may multiply or join with other amoeboid cells to form a plasmodium.
- As the plasmodium develops, it releases the enzyme **Glucosinolase**. This enzyme converts glucobrassicin already present in the host cell into the plant hormone, **IAA**.

- It causes the host cells to enlarge up to 20 times of its normal size. This increase in the cell size makes the aerial parts allocate more nutrients to the root. These nutrients are consumed by the pathogen.
- As the plasmodium grows, it divides and infects neighboring cells causing them to enlarge. Clusters of these enlarged cells are responsible for the clubbing on the roots and are referred to as 'Kankheitsherd'. These Kankheitsherd are diagnostic of *P. brassicae* and can be observed in cross sections of infected roots.
- Not all amoeboid cells infect cortical cells. Some move into the vascular tissue and infect the cambial cells of the host. The soft undeveloped cell walls of the cambial cells allows *P. brassicae* to easily travel up and down the root, infecting cortical cells, vascular ray cells, and cambial cells as it goes. The infection and resultant swelling of the vascular ray cells is responsible for the characteristic wilt symptoms associated with *P. brassicae*. As the ray cells swell to abnormal sizes, sections of xylem are pushed aside, and the continuity of the water column is broken.
- The way in a secondary heterokaryotic plasmodium is formed is not known for sure. The formation and position of the diploid phase in the life cycle is unclear (Webster, 2006). Karyogamy probably occurs at a late stage of infection, which is the sexual stage. It results into diploid plasmodia.
- The diploid plasmodium quickly undergoes meiosis and develops into resting cysts. These new cysts will be released into the soil as other soil microorganisms decompose the club root.
- *P. brassicae* overwinters as resting spores. When environmental conditions are favorable, a resting spore germinates and produces one zoospore which infects a host root hair and produces a plasmodium within the root.

Control Measures

1. **Field Selection:** When possible, select fields with no prior history of club root for crucifer production. Fields should also be well drained and have a soil pH of 7.2 or greater.
2. **Crop Rotation:** A 7 to 10 year rotation out of crucifers is recommended. Some researches have also found that a rotation to cereals is most effective in reducing the amount of inoculum present.
3. **Use Disease Free Transplants.**
4. **Lime Soil to Increase pH:** This management strategy may involve large amounts of lime, for instance 700 KG/Acre is necessary to increase the pH from 7.0 to 7.2. In addition, increasing soil pH may limit rotation options by directly affecting subsequent crops or by increasing the likelihood of disease development on those crops (eg. scab on potato).
5. **Do not use tail water from infected fields on non-infected fields.**
6. **Resistant Varieties:** Limited resistance is available to commercial growers of cabbage, turnips, radishes and rutabagas. Resistance is race specific.
7. **Fungicide Application:** PCNB is labeled for control of club root and may be soil incorporated at planting, used as a plant dip or applied in transplant water. Crops included on the label are: broccoli, Brussels sprouts, cabbage, Chinese cabbage (lightheaded varieties), cauliflower, collards, kale and mustard greens.

Myxomycota (Slime Molds)

Introduction

Slime molds are highly unique group of achlorophyllous, true nucleated heterotrophic organisms which live on the decaying moist lignicolous substratum in plasmodial or the fruiting forms of varied colours in nature.

They share the significant characters with both animals and fungi. The somatic structure is devoid of cell wall.

The sporophytic phase is diploid, multinucleated, creeping, naked protoplasm, surrounded by plasma membrane and a gelatinous layer which resembles the amoeboid structure of protozoans.

Distinction from Eumycota

When the first slime moulds were described by Johann H. F. Link in 1833, they were given the term myxomycetes (Gr. myxa = slime). Link used the suffix -mycetes because of the superficial similarity of the fructifications of slime moulds with the fruit bodies of certain fungi, notably Gasteromycetes.

It has been accepted widely now that slime moulds lack any true relationship with the Eumycota.

Slime moulds differ substantially from the Eumycota not only in phylogenetic terms, but also regarding their physiology and ecology. Their vegetative state is that of individual amoebae in the cellular slime moulds, or of a multinuclear (coenocytic) plasmodium in the plasmodial slime moulds. Motile stages bearing usually two anterior whiplash-type flagella may be present in the plasmodial slime moulds and in the Plasmodiophoromycota. Amoebae or plasmodia feed by the ingestion (phagocytosis) of bacteria, yeast cells or other amoebae. This is followed by tally different from extracellular degradation and absorption as shown by Eumycota.

Numerous phylogenetic analyses of DNA sequences encoding rRNA molecules and various structural proteins or enzymes have suggested that the Dictyosteliomycetes, Protosteliomycetes and Myxomycetes are related to each other whereas the Acrasiomycetes have a different evolutionary origin (Baldauf, 1999; Baldauf et al., 2000). However, these four groups are certainly distant to the Eumycota.

Therefore, the Myxomycota has now been kept out of Eumycota and now treated under Protista.

Affinities

Similarities with animals

1. The vegetative thallus is like animals i.e. achlorophyllous, devoid of definite cell wall and surrounded by plasma membrane.
2. They produce amoeba like irregular thallus (cellular slime molds) or multinucleated coenocytic plasmodium (acellular slime molds).
3. The amoeboid structures may or may not develop loco-motory organs i.e. two anterior whiplash flagella.
4. The mode of nutrition is by ingestion i.e. by producing pseudopodia or plasmodial out growths around the food particles, engulfing and digesting them intra cellularly in vacuoles same like in protozoans. This process of ingestion is called as phagocytosis.

Similarities with Fungi

1. They are achlorophyllous, true nucleated heterotrophs.
2. Slime molds inhabit the moist decaying lignocellulosic substratum like fallen leaves, twigs, wooden logs, decaying animal dung, sometimes on the surface of garden plants tree trunks, and act as decomposers of complex polysaccharides into simple sugars like fungi.
3. Slime molds somatic structure is coenocytic as in the coenocytic fungi.
4. Slime molds plasmodium (vegetative thallus) absorbs and assimilates its nutrition in the soluble form by direct contact of the plasma membrane with substratum.
5. Slime molds possess reserve food material as fat droplets and glycogen particles.
6. They reproduce asexually by amoeboid cells (motile or non motile) which during favourable conditions divide mitotically several times and parenate as microcysts or spherules during unfavourable conditions and remain viable for very long period in the soil.

7. They produce sexual meiotic spores in the fruiting bodies. The Spores are thin walled, hyaline surrounded by definite cell wall of cellulosic in nature but not chitinous as in other fungi and mode of dispersal is like fungi by wind, water and by insectal movements.
8. The pattern of life cycle slime molds follow is same like fungi.
9. Slime molds produce sclerotia during unfavourable conditions same like fungi.

Difference from fungi

1. Mode of nutrition – Ingestion (Phagocytosis) and extra cellular degradation and absorption.
2. Absence of definite cell wall around somatic structures.
3. Plasmodium shows cytoplasmic streaming.

Recently proposed systematic position

Alexopoulos, C.J., Mims C.W. and Blackwell, M. 1996

- Kingdom – Protista
- Phyla:
 - Plasmodiophoromycota
 - Dictyosteliomycota
 - Acrasiomycota
 - Myxomycota

Webster J. and Weber R.W.S. 2007

- Kingdom – Protozoa
- Phylum – Myxomycota
- Classes:
 - Acrasiomycetes
 - Dictyosteliomycetes
 - Protosteliomycetes
 - Myxomycetes

Important groups of Myxomycota

Acrasiomycetes

The trophic stage consists of amoebae which are morphologically distinct from those of the dictyostelid cellular slime moulds in having a cylindrical, rather than flattened, body bearing a single large-lobed (lobose) anterior pseudopodium. The granular cellular contents trail behind the pseudopodium, which appears clear. The posterior end is knob-shaped and is called the uroid.

Acrasid slime moulds are common on decaying plant matter, in soil, on dung and on rotting mushrooms, but they are rarely recorded because of their small size.

Spore-bearing structures are called sorocarps.

Dictyosteliomycetes

The Dictyosteliomycetes are a group of cellular slime moulds comprising 46 species in four genera (Kirk et al., 2001). The best-known example is *Dictyostelium*.

The life cycle of *D. discoideum* is shown in Fig. 1. Amoebae of dictyostelids are morphologically different from those of acrasids in that they have filose (acutely pointed) rather than lobose pseudopodia. Each spore from a sorocarp germinates to give rise to one uninucleate haploid amoeba which feeds by phagocytosis of bacteria. Amoebae reproduce asexually by division to form two haploid daughter amoebae.

The most striking feature of *D. discoideum* is the aggregation of thousands of amoebae to form a pseudoplasmodium with radiating arms. This is a vegetative process not involving meiosis or mitosis.

Myxomycetes: true (plasmodial) slime moulds

The Myxomycetes are by far the largest group of slime moulds, comprising some 800 species in 62 genera.

The vegetative phase is a free-living plasmodium, i.e. a multinucleate wall-less mass of protoplasm. This may or may not be covered by a slime sheath. Plasmodia vary in size and can be loosely grouped into three categories.

1. Protoplasmodia are inconspicuous microscopic structures usually giving rise only to a single sporangium. They resemble the simple plasmodia of protostelids.

2. **Aphanoplasmodia** (Gr. aphanes = invisible) are thin open networks of plasmodial strands. The aphanoplasmodium is transparent, with individual strands only 510 mm wide and the entire plasmodium about 100200 mm in diameter. Most aphanoplasmodia are only seen with the aid of a dissection microscope.
3. **Phaneroplasmodia** (Gr. phaneros = visible) are large sheets or networks with conspicuous veins within which the protoplasm shows rhythmic and reversible streaming.

The life cycle of *Physarum polycephalum*, a typical myxomycete, is summarized in Fig. 2.

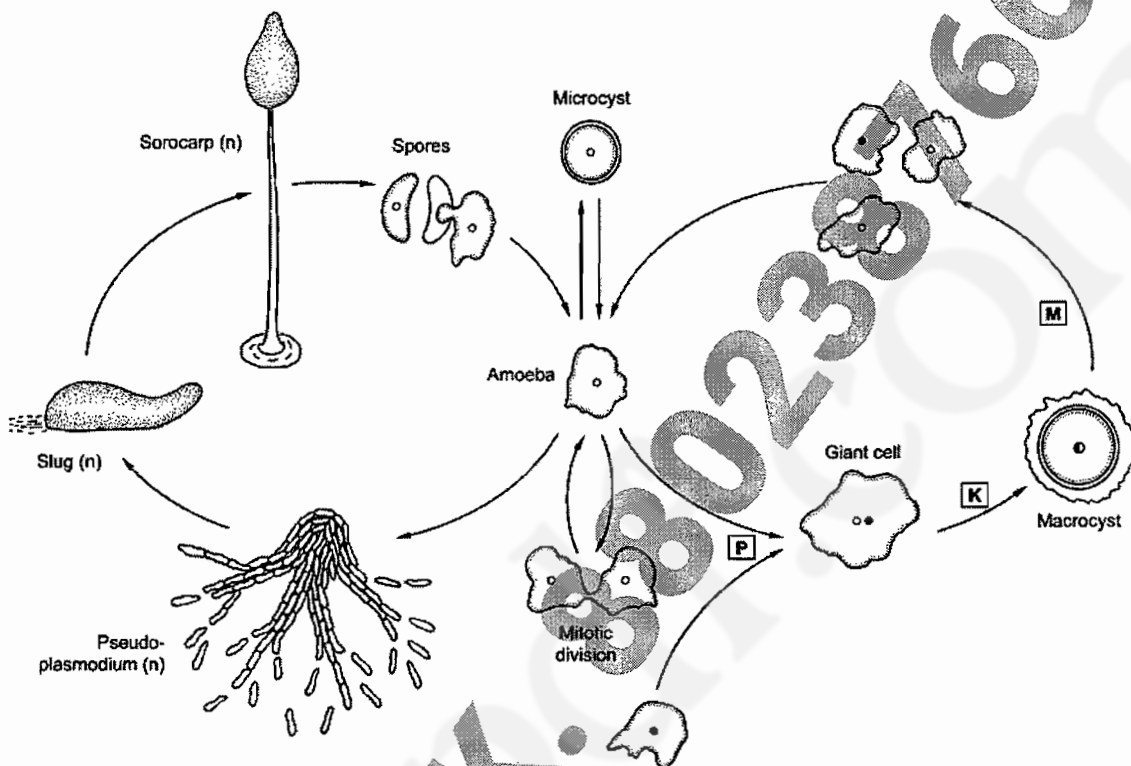


Figure 1: Life cycle of *Dictyostelium discoideum*. The central feature is the haploid amoeba which is free-living in the soil. It divides mitotically to produce two daughter amoebae or, under unfavourable conditions, may form a microcyst. If two amoebae of compatible mating type meet, a diploid macrocyst may be formed which can remain dormant for some time and eventually germinates by meiosis and then mitosis to release numerous haploid amoebae. Under certain circumstances, starvation may lead to aggregation of amoebae to form a slug and a sorocarp in which individual amoebae become converted into spores. These are purely asexual, and meiosis is not involved in their formation or germination. Open and closed circles represent haploid nuclei of opposite mating type; diploid nuclei are larger and half-filled. Key events in the life cycle are plasmogamy (P), karyogamy (K) and meiosis (M).

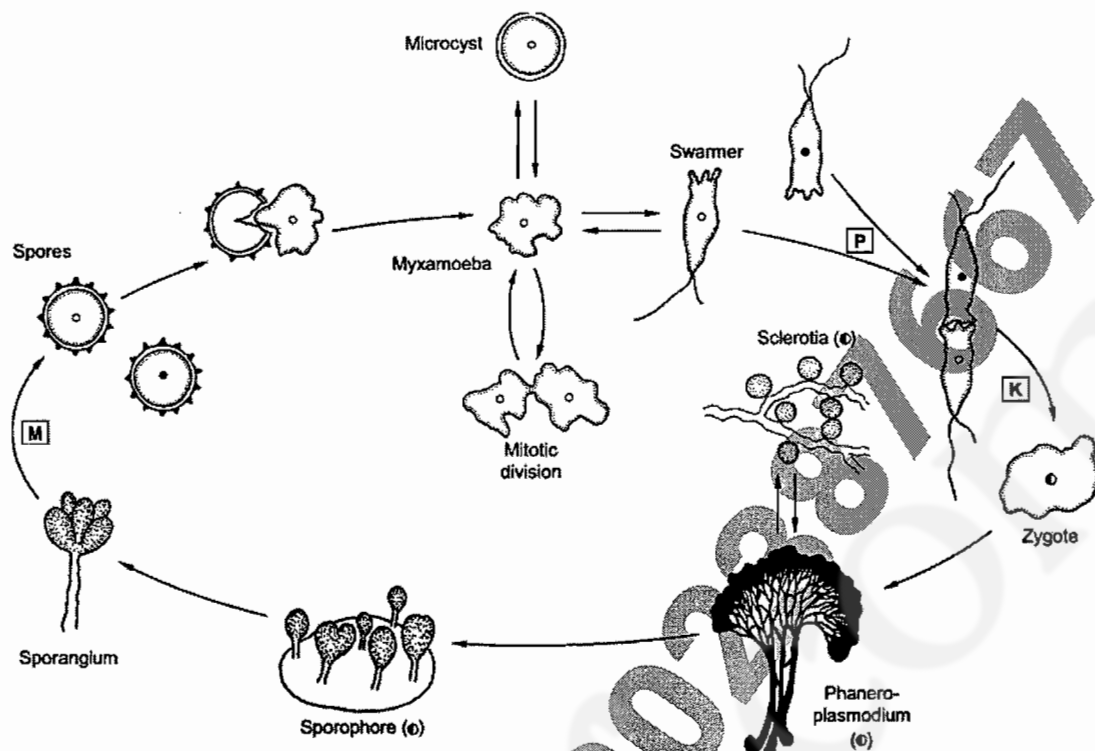


Figure 2: Life cycle of the myxomycete *Physarum polycephalum*. Spores released from the sporangium are haploid and can germinate by releasing either a single myxamoeba or a swarmer cell. These two cell types are interconvertible. The myxamoeba can divide mitotically. In *P. polycephalum*, plasmogamy (P) usually takes place between swarmer cells which must belong to different mating types. Karyogamy (K) follows, and the diploid zygote establishes a phanero-plasmodium. When nutrients become limiting, a sporophore is formed and differentiates sporangia in which meiosis (M) occurs. Unfavourable conditions can be overcome at the haploid stage when the myxamoeba forms a microcyst, or at the diploid stage when the plasmodium forms sclerotia. Open and closed circles represent haploid nuclei of opposite mating type; diploid nuclei are larger and half-filled.

Economic importance

Slime molds in general have little direct economic importance. The colourful plasmodium and fruiting bodies of slime molds are beautiful and the delicately constructed intricate designs have attracted the photographers and artists to exhibit them in the form of paintings, sceneries and photographs e.g. the creamish yellow coloured fruiting bodies of *Physarum polycephalum* and the grayish black linear hair like fruiting bodies of *Stemonitis* sp growing on woods.

- Ecologically these are important in the food web as food for insects.
- They can be used as an edible protein source in future. The young fruiting bodies of *Enteridium*, *Lycoperdon* and *Fuligo septica* are eaten in Mexico.
- The cultures of slime molds are used in cell and molecular biology e.g. *Physarum polycephalum* and *Didymium iridis* cultures are used to study the genetic variability in nature and in understanding the process of ageing and how it can be controlled both by the nucleus and cytoplasm (cell longevity).
- Some antibiotics have been isolated from *Physarum gyrosom* which can be used against yeasts, gram (+) and (-) bacteria.
- Slime molds, wherever they grow, utilize all kinds of spores of microbes as food and do not allow any microbe to colonise that substratum are considered as a purest form of protoplasm found in nature. It is frequently employed in research to understand the chemical composition of protoplasm.
- These are used as ideal experimental tools in research laboratories to understand the various processes in living beings i.e. mitotic cycle, morphogenesis, physiology, the chemical changes which govern reproduction, the structure and movement of protoplasm etc. e.g. *Physarum polycephalum* is used in cancer research and *Dictyostelium discoideum* (Acridiomycete) in morphogenesis.

Parasexuality

The process of sexual reproduction involves the union of two compatible nuclei (karyogamy) followed by meiosis. Meiosis brings about recombination of genetic material, leading to the emergence of new individuals bearing some new hereditary characters. However, there are a great number of fungi, which do not go through sexual reproduction and still they produce new individuals bearing some new hereditary characters. It is probably due to this, that a large number of new species and genera are frequently described even among the non-sexual fungi.

It is now established that there exists a device of sexual bypass, called **parasexual-reproduction** or **parasexuality**, which is responsible for genetic recombination even in the fungi which do not enter into true sexual cycle. Besides, there are reports that parasexuality also operates in some such fungi, which simultaneously enjoy sexual reproduction.

Parasexuality can be defined as a phenomenon in which the three processes, e.g., plasmogamy, karyogamy and haploidization occur at an unspecified time at unspecified points in the life of a fungus and eventually establish non-parental type (i.e. recombinant) nuclei even without the true sexual process ever occurring. The process of haploidization occurs without involving meiosis.

The parasexual cycle thus demonstrates that the genome can become reorganized without passing through meiosis. Sexual and parasexual cycles provide quite different processes leading to a similar end result.

G. Pontecorvo and J.A. Ropper (1952) for the first time discovered parasexuality in *Aspergillus nidulans*, the imperfect stage of *Emericella nidulans*. Later on, Pontecorvo (1956) continued his studies on parasexuality and discovered several steps involved in the process, which are described below.

1. **Formation of heterokaryotic mycelium:** A heterokaryotic mycelium means presence of two types of nuclei in the mycelium of a fungus. It may come about by mutation of a nucleus within a mycelium or by fusion of mycelia with different types of nuclei. Somatic incompatibility is a deterrent to establishing heterokaryons in nature and these nuclei are rare in nature. However, in the laboratory, appropriate mutant strains can be manipulated routinely to create heterokaryons.
2. **Karyogamy and multiplication of diploid and haploid nuclei side by side:** Once heterokaryons are established, there is a possibility for the parasexual cycle to take place. The next step is the fusion between the two different types of nuclei and formation of a diploid nucleus. Once again, this is an extremely rare event to occur in nature. Nevertheless, the laboratory manipulations can induce the different nuclei to fuse together. After karyogamy, the fungal hypha will have some diploid and some haploid cells. Both these types of cells would proliferate on their own, creating haploid and diploid sectors within the same mycelium.
3. **Mitotic crossing-over during multiplication of heterozygous diploid nuclei:** In many fungi and some animals, mitotic crossing over is a well-recorded phenomenon (First reported by Curt Stern in *Drosophila* in 1931). It takes place when homologous chromosomal segments are accidentally paired in asexual cells. During mitotic crossing over, chromosomal segment exchange occur just the way they do in the meiotic prophase-I. Thus, recombinant chromosomes are created due to mitotic crossing taking place during mitotic divisions of the diploid nuclei.
4. **Haploidization:** It has been observed that the diploid nuclei formed after heterokaryosis in the above-described manner are usually not very stable. The resultant nucleus being unstable, the chromosomes are lost during subsequent mitotic divisions. The haploid state is finally attained by random chromosome elimination. Commonly, because of mitotic crossing over having taken place earlier, and random loss of chromosomes, the resultant haploid cell is genetically different from the parents. This may lead to a different offspring, but the numbers of possible recombinations is reduced.

Thus, parasexual cycle gives the advantage of recombination while being limited to the genetic material already in the thallus and totally avoiding meiotic division.

It was once thought that the parasexual cycle is important in the members of Deuteromycotina because it is associated with the recombination of genes without sexual process. However, the current view is that these instances are rare in nature (Deacon, 2005).

Moreover, while sexual reproduction is an event precisely controlled by the host genome, the parasexual cycle appears to be uncommon and uncontrolled. This is mainly because, segregation following meiosis leads to controlled separation of chromatids into nuclei, whereas in the parasexual the haploid state is attained by random loss of chromosomes.

The parasexual cycle is less efficient than the sexual cycle, but it can be of value when enormous numbers of offspring are placed under selection pressure. The advantage is due to the slight variation attained from parasexual recombination.

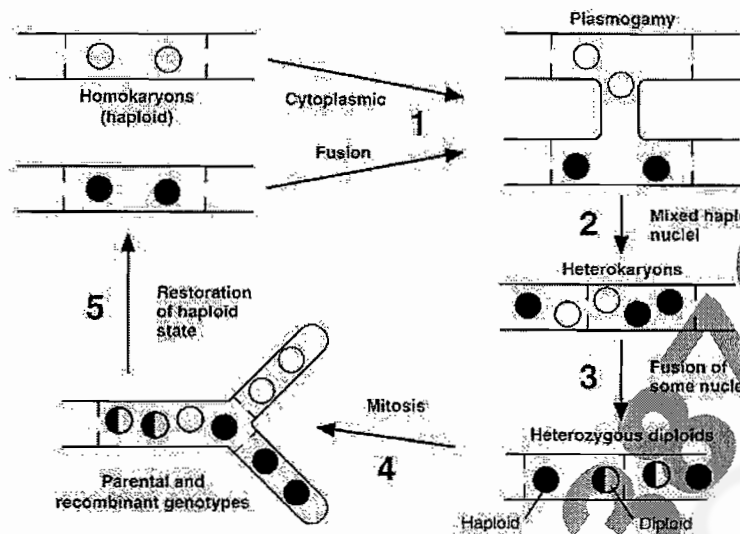


Figure 1: Parasexual cycle events

In the recent years, the parasexual system has emerged as the easiest and most efficient tool for genetic manipulation of many biotechnically important zygomycetous fungi, including the genera *Absidia* and *Thamnidium*, employed for steroid hydroxylation, and *Blakeslea trispora*, industrially exploited for large scale production of β -carotene.

Heterothallism in Fungi

Introduction to Heterothallism

Heterothallism, with reference to the fungi, is a phenomenon in which sexual fusion is not possible between the thalli of identical genetic types. Rather, sexual fusion strictly occurs between different sexual strains within the same species – which are regarded as Mating Types. This process, by blocking sexual reproduction between genetically identical types, promotes heterozygote formation, thereby increasing the chances of genetic variations within the population. In this sense, heterothallism has been regarded as an advanced type of sexual behaviour.

Discovery

Heterothallism was discovered in 1904 by the A.F. Blackeslee, when he observed that in most members of Mucorales two genetic types of thalli were present, although there were morphologically similar. The zygosporangium formation took place only when two different types of thalli were growing close by. When he isolated sporangiospores from a single sporangium and allowed them to germinate and form colonies of identical types of thalli, he noted that there was no zygosporangium formation. On the other hand, when the sporangiospores from two different types of thalli were collected, mixed and then allowed to germinate and establish a colony containing two different types of thalli, the zygosporangia readily formed.

Subsequent studies revealed that multiple mating types and heterothallism prevail in several fungal groups.

Basic Types of Heterothallism

1. **Morphological Heterothallism:** The term applies when within the species, a sexual dimorphism exists, that is male and female thalli are separate. In such cases, the only mode of sexual fusion that is possible is between two different thalli. This type of heterothallism is seen in *Achlya ambisexualis*, *Blastocladiella variabilis*, *Phytophthora palmivora* and *Peronospora parasitica*.
2. **Physiological Heterothallism:** Here, either the thalli are sexually undifferentiated or they are bisexual. Nevertheless, sexual fusion is not permitted within the same thallus. Examples include *Saccharomyces*, *Neurospora*, *Rhizopus*, *Mucor*, *Puccinia* etc.

Occurrence of Heterothallism

Fungi of different groups display heterothallism and different mating types. Some important examples are:

1. **In Chytridiomycetes:** *Blastocladiella* sp.
2. **In the Oomycetes** (no longer considered true fungi): *Achlya*, *Phycomyces nitens*, Some species of *Phytophthora*, *Pythium* and *Peronospora*.
3. **In Zygomycotina:** *Rhizopus*, *Mucor*, *Zygorhynchus*
4. **In Ascomycotina:** The yeast *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*, *Ascobolus magnificus* and *Ascobolus carbonarius*
5. **In Basidiomycotina:** In *Puccinia*, Several species of *Ustilago*, *Coprinus cinereus*, Some species of *Agaricus*.

Operation of Heterothallism

The operation of heterothallism can be illustrated through the following examples.

1. **Rhizopus:** During sexual reproduction, compatible strains (+ and -) form short, specialized hyphae called gametangia. At the point where two complementary gametangia fuse, a thick-walled, spherical zygosporangium develops. The zygosporangium typically contains a single zygosporangium. Nuclear karyogamy and meiosis (sexual recombination) occur within the zygosporangia, which are thought to be long-lived and resistant to adverse conditions. They may germinate to form hyphae or a sporangium.
2. **Saccharomyces:** The yeast *S. cerevisiae* has a simple mating system, with cells of two haploid mating types termed a and α . These cells can conjugate to form a diploid cell containing both a and α information. The a/α diploid is not capable of mating but can initiate meiosis to form four haploid products, two of which are mating type a and two of which are mating type α . Laboratory strains typically have stable mating types, and are termed heterothallic. In contrast, most wild strains are homothallic, and do not have stable mating types. Instead, during mitotic growth the cells are capable of switching their mating types.

3. *Neurospora crassa*: *Neurospora crassa* is heterothallic and requires two mating types, A and a, for sexual reproduction. The mating-type loci, mat A and mat a, control gene expression required for the mating process. The DNA sequences at the mating loci of opposite mating types are very different, and are therefore regarded as 'idiomorphs' as opposed to alleles.
4. *Coprinus cinereus*: The basidiomycetous fungi are novel in that they have multi-allelic mating-type genes. In *Coprinus cinereus*, more than 12000 mating types exist, demonstrating the diversity in mating systems and complex levels of control within the filamentous fungi. *Coprinus* has two unlinked mating-type loci, A and B, which are polymorphic and contain sub-loci called α and β .

Genetic Basis of Heterothallism

With regard to genetic regulation of heterothallism, two variations are observed:

1. **Bipolar heterothallism**: In several *Neurospora* species, *Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, the mating type corresponds to two allelic forms of a single locus and, accordingly the heterothallism is known as bipolar. It is the more common type of heterothallism among the fungi. It has been proposed recently by Tatiana Giraud *et al* (in the journal *Eukaryotic Cell*, May 2008) that all heterothallic ascomycete fungi present a bipolar system.
2. **Tetrapolar heterothallism**: In this type of fungal mating system, compatibility is determined by alleles at two unlinked loci. It is thus called tetrapolar heterothallism. It is common in Basidiomycetous fungi. For example, in *Ustilago maydis*, cell recognition and fusion are regulated by a pheromone-receptor system that resides at the *a* locus, and persistence of the resulting dikaryon, i.e., hyphae with the two haploid nuclei remaining separate in the cells, is determined by heterozygosity for the second mating-type locus, the *b* locus.

Molecular Basis of Heterothallism

In fungi, mating compatibility is regulated strictly in the haploid stage by specialized regions of the genome known as the mating-type loci (MAT). The genes located at MAT locus are known to encode:

1. **Transcriptional activator protein**, which further stimulates the synthesis of signaling substances and receptors for recognition system (such as a pheromone-receptor system);
2. **DNA binding structural proteins**, which provide stability of the heterozygotes.

Importance of Heterothallism

Sexual reproduction with heterothallism is of fundamental importance as it creates a dimorphic pair of genetic material thereby bringing in genetic variation. This variation is important for evolution because it can introduce new combinations of genes every generation and new features, some of which can increase fitness of the individuals. Without genetic variation, some of the basic mechanisms of evolutionary change cannot operate.

Mycorrhiza

Introduction

The word – “mycorrhizae” – literally means “fungus-roots”. Mycorrhiza is a symbiotic relationship between fungal hyphae and roots of higher plants.

About 95% of the world’s land plants form the mycorrhizal relationship in their native habitats. It is estimated that mycorrhizal fungal filaments explore hundreds to thousands more soil volume compared to roots alone. This symbiotic relationship provides the fungus with a renewable source of food through access to organic nutrients.

Discovery

They were discovered by the German botanist A.B. Frank in 1885.

Types/Forms of Mycorrhizae

Mycorrhizal type	Typical host plants	Fungi involved	Major significance
Arbuscular mycorrhizas	Many	Glomeromycota	Phosphorus uptake from soil
Ectomycorrhizas	Forest trees, mainly in temperate and boreal regions	Basidiomycota, Ascomycota	Nitrogen uptake from soil
Ectendomycorrhizas	Mainly pines, spruce, and larch	Ascomycota of the genus <i>Wilcoxina</i>	Mineral nutrient uptake from soil
Arbutoid mycorrhizas	<i>Arctostaphylos</i> , <i>Arbutus</i> , <i>Pyrola</i>	Basidiomycota, similar to ectomycorrhizal fungi	Mineral nutrient uptake from soil
Monotropoid mycorrhizas	Nonphotosynthetic plants, e.g. <i>Monotropa</i>	Basidiomycota such as <i>Boletus edulis</i>	Plants obtain sugars from ectomycorrhizal fungi attached to trees
Ericoid mycorrhizas	Heathland plants. <i>Erica</i> , <i>Calluna</i> , etc.	Ascomycota and mitosporic fungi; <i>Hymenoscyphus ericae</i>	Nitrogen uptake from soil
Orchid mycorrhizas	Orchids	<i>Rhizoctonia</i> -like fungi (basidiomycota)	Fungi supply the plant with sugars

Table 1: The major types of mycorrhiza and their ecological significance

- Arbuscular or Vesicular-Arbuscular (VAM)** – (i) Most common form. (ii) Approx. 75% of all angiosperm and gymnosperm genera are thought to form AM under appropriate conditions. AM fungi may also be found in some ferns, mosses and liverworts. (iii) The host genera include most plants important in agriculture, forestry and horticulture. (iv) Few angiosperm families lacking mycorrhizae include Brassicaceae, Caryophyllaceae, Chenopodiaceae, Crassulaceae, Cyperaceae, Juncaceae, Pinaceae, etc. (v) AM is an endomycorrhiza, which means the fungus enters the cells of the root. After the entry of the hyphae into the plant cells, they produce balloon-like (vesicles) or dichotomously-branching invaginating (arbuscules) structures. (vi) AM are found on grasses, most crop plants, many trees, shrubs, flowers, and 80% of the world’s species. (vii) AM formed only by fungi in the class Glomeromycetes of phylum Zygomycota and include genera like *Glomus*, *Paraglomus*, *Gigaspora*, *Sclerocystis*.
- Ectomycorrhiza (EM)** – (i) Fungus enters the root but not the root cells. (ii) Most trees of temperate northern hemisphere forests form EM, and for these trees the fungi are obligately associated with the survival of the host. (iii) Trees are found in families including Betulaceae, Fagaceae, Oleaceae, etc. (iv) Most of the fungi come from the Holobasidiomycota and Ascomycota and a few from the aseptate Endogonales. Such as *Boletus*, *Amanita*. (v) These fungi form a thick sheath around the terminal absorbing root of the host plant. This sheath is called Mantle. (vi) In the root cortical region, the fungus forms a mycelia network called the Hartig’s Net.
- Ectendomycorrhiza** – (i) Fungus enters the root cells. (ii) Host plants belong to families like Asteraceae, Campanulaceae, Casuarinaceae, Euphorbiaceae, Fabaceae.

4. **Arbutoid Mycorrhizas** – (i) Look like EM and have similar fungi, but are technically endomycorrhizas. (ii) Manzanita, madrone, and a few other plants form this type. (iii) Mostly members of basidiomycota form arbutoid mycorrhizas. (iv) There is penetration of the host cell but at the same time there is formation of Mantle and Hartig's Net as in EM. (v) Within the host cell, the fungus forms coil like structures.
5. **Monotropoid** – (i) Found on certain plants without chlorophyll, such as *Monotropa*. (ii) Traditionally called saprophytic, they share a mycorrhizal fungus with a nearby tree and thus parasitize nearby tree. (iii) Fungi forming this association are mostly from two genera, viz., *Boletus* and *Armillaria*.
6. **Ericoid Mycorrhizas** – (i) Formed by blueberries and related plants. (ii) Few members of Holobasidiomycetes and many Ascomycota form this type – families include Ericaceae and Epacridaceae. (iii) There is a penetration of the host cell where the fungus forms coil like structures. (iv) But there is no formation of Mantle and Hartig's Net as in EM.
7. **Orchid Mycorrhizas** – (i) Unique in that they are required for seed germination. (ii) Some kinds of orchids never photosynthesize, but instead parasitize the mycorrhizal fungus. (iii) Most orchids associate with members of the form genus *Rhizoctonia*. A few achlorophyllous orchid species have been recorded to associate with a variety of other genera, which may also form ectomycorrhizas or are known wood-rotting fungi or pathogens. (iv) Seeds of orchids are undifferentiated, and lack significant reserves of nutrients. Germination depends on colonisation by a specific mycorrhizal fungus. (v) The fungus forms a tight coil or peleton following invagination of the plasmamembrane and extension into the cell (in undifferentiated embryo of the seed). (vi) Orchid mycorrhizas are different from other types of mycorrhizas in the nature of the nutrient exchange. After establishment of mycorrhiza, organic carbon and other nutrients are passed from the fungus to the seed. The fungus continues to supply the protocorm with all its organic energy until plants start to photosynthesize.

Mycorrhizae in India

The rapid developments in agriculture with increasing use of chemical fertilizers and pesticides have caused pollution of soil, subsoil water and environment. Increasing number of scientists are, therefore, becoming involved in research on biofertilizers mainly on mycorrhiza (a fungal – root association) to not only alleviate chemical pollution but also to save on chemical fertilizers, reduce the use of pesticides and to boost agriculture production. The mycorrhizal research has attracted universal attention and has made remarkable progress. Over 400 scientists in India are presently engaged in mycorrhiza research.

TERI, with support from the Department of Scientific and Industrial Research, Government of India, initiated a project, titled – Design and Development of On-line Database on Mycorrhiza.

The objectives are to – (i) collect literature, build an on-line searchable database, digitize and store abstracts and bibliographies; (ii) develop methodologies for retrieval of information; (iii) develop a directory of mycorrhiza scientists in Asian region particularly India to create a network of scientists as also centers and institutions where mycorrhizal research is being carried out; (iv) facilitate open access to current research findings and development; promote research among scientists, agriculturists, mycorrhizologists and students.

Advantages to the plants from Mycorrhizae

1. Improved nutrient and water uptake due to the mycelium's tremendous surface area to absorb water and mineral nutrients from the soil. Mycorrhizal fungus explores a larger volume of soil than root systems at a lower cost to the plant. The mechanisms of increased absorption are both physical and chemical. A role in mineral acquisition is most clearly seen in case of phosphorus ions, which are tightly bound to iron oxides in many soils. Plant roots are generally incapable of accessing these phosphorus sources.
2. Improved root growth.
3. Improved plant growth and yield in a number of commercially important plants. Egs – Alfalfa, Citrus, Coconut, Cotton, Rice, Wheat, etc.
4. Reduced drought stress because the host root has greater access to soil water reserves.
5. Parasitic nematode control by the formation of thick mantle which acts as a physical barrier outside the host root.
6. Disease Resistance – Other parasite control by release of several anti-microbial chemicals such as Polyacetylenic acid and Diatretyne nitrile around the host root. Polyacetylenic acid protects the conifer root from *Phytophthora* attack.
7. Reduced transplant shock.

8. In forest ecosystems the mycorrhizae are critical in establishing Nurse Plant relations, in which the mycorrhizal fungus establishes a nutritional relation between the host plant and a nearby tree. Thus the host plant is in effect a parasite of the tree by way of the mycorrhizal fungus. This association plays a vital role in the survival of many seedlings on the forest floor.
9. They produce organic glues that make the soil more clumpy and porous, improving its structure and resiliency.
10. Some of the earliest fossil plants show evidence of mycorrhizas associated with them which indicates that the mycorrhizae were crucial in early colonization of the land habitat.
11. Plants grown in sterile soils and growth media often perform poorly without the addition of spores or hyphae of mycorrhizal fungi to infect the plant roots and aid in the uptake of soil mineral nutrients.

Mycorrhizae in forest ecosystems

Mycorrhizal associations predominate in most natural terrestrial ecosystems. Whereas the AM fungi are widespread geographically and have a very extensive host range, the ECM (ectomycorrhizas) fungi are more restricted, forming associations predominantly with genera of important woody plants. Nevertheless, ECM fungi are dominant components of the ground-dwelling macro-fungi in ecosystems where members of the following plant families abound: Betulaceae, Dipterocarpaceae, Fagaceae, Myrtaceae, Pinaceae, Ulmaceae, Salicaceae. ECM fungi are common in tropical forests of Asia but are uncommon in many forests in Africa and South America. In Asia, the number of host species tends to increase with altitude and at higher latitudes.

Roles in ecosystems

In nature, the situation is far more complex as a single tree may have fungal partners which can vary in time and space. In forest ecosystems, the mycorrhizae are critical in establishing NURSE Plant relations in which the mycorrhizal fungus establishes a nutritional relation between the host plant and a nearby tree. Thus the host plant is in effect parasite of the tree by way of the mycorrhizal fungus. This association plays a vital role in the survival of many seedlings on the forest floor. The study by Moyersoen et al., (1998), on the co-occurrence of AM and ECM fungi in rainforest in Cameroon, provides a good example of a field study exploring possible functional roles of mycorrhizal fungi.

Carbon transport

The fungal/plant interface provides a conduit for the movement of carbon from the plant to the fungus, and for movement between plants linked by mycelia. It is generally believed that mycorrhizal plants direct more of their photosynthates into the soil than nonmycorrhizal plants. This extra carbon accumulates in patches and at the edge of hyphal mats, and boosts the energy supply to the detrital food web, benefiting saprophytic microbes and other soil organisms. Because the chemical and physical environment around mycorrhizas (the mycorrhizosphere) differs from nonmycorrhizas, presumably it provides microhabitats for soil biota that are not present in the rhizosphere of nonmycorrhizal roots. Mycorrhizal fungi are estimated to consume from 15 to 50% of net primary production.

Nutrient cycling and nutrient conservation

Fungi are crucial components of ecosystems as they transport, store, release and cycle nutrients. In forests, litter is an important nutrient reservoir. ECM fungi can mobilise P, N and other nutrients from litter to tree roots. It was estimated that ECMs account for 43% of the annual turnover of N in a *Pseudotsuga menziesii* forest in Oregon.

Soil structure

It is obvious from the examination of ECM mycelial mats, that mycorrhizal fungi have a big impact on soil structure. In agricultural soils, AM fungi increase the formation of soil aggregates.

Food for animals

Long-distance dispersal of spores from ECM fungi with hypogaeal (truffle-like) sporocarps depends largely on mammal mycophagy. Mycophagy is widespread and has been demonstrated in Europe, Australasia and North America. Mycophagy serves to maintain populations of ECM fungi and provides nourishment to small mammals. Sporocarps are good sources of water, protein, carbohydrates and minerals. The tripartite relationship between truffles/truffle-like fungi, vertebrates such as squirrels and many ground-dwelling marsupials, and the host trees, are well known. Less well known is the role that mycorrhizal fungi play as a food source for invertebrates and the role of invertebrates in dispersal of ECM and AM fungal spores.

Lichens

Introduction

Lichens are autotrophic, slow growing, superficial, perennial, long-lived synthetic or composite (dual) plants which possess a symbiotic association of an alga and a fungus. The algal partner is called **phycobiont** which helps in photosynthesis and sometimes in nitrogen fixation while fungal partner, called **mycobiont**, is dominant and helps in fixation, protection, reproduction, and absorption of water and some mineral salts.

Theophrastus, the father of Botany, in his book – ‘History of Plants – used the term lichens for extra plant growth on the bark of trees.

The credit for the discovery of lichens goes to Tulsane (1852). Reinke (1872) distinguished this association between algal and fungal partner as consortium.

Habit and distribution

There are about 400 genera and 16000 species of lichens, widely distributed in most parts of the world and found in varied habitat from arctic to Antarctic and all regions in between. They are common in tropical rainforests than temperate regions. They grow in polluted and SO₂ free area on old walls, window panes, tree trunks as epiphytes, decaying wood, leaves, barren rocks, stones, roofs, cooled volcanic lava, icy tundra, alpine soil, on siliceous rocky sea shores (e.g., *Peltigera*, *Caloplaca*) and on hard rocks of fresh water (e.g., *Hymenelia*). They require – (i) pure air free from SO₂ and pollution, (ii) cold humid climate, (iii) ample light.

In India, they are common in Eastern Himalayas at 4000–10000 ft and Western Himalayan region. Darjeeling, Sikkim, Gangtok are ideal places for lichen collection.

Factors responsible for worldwide distribution of lichens – (i) Symbiotic nature, (ii) Profile methods of vegetative propagation by propagules like soredia and isidia, (iii) Efficient means of dispersal, (iv) Resistance to tolerate high temperature and dessication, (v) Xerophytic nature.

They grow at very slow rate due to non-availability of – (i) food at sites where lichens grow, (ii) low light intensity on trees, walls and shaded rocks, (iii) cool temperature and moisture for maximum period.

According to habitat, lichens are grouped into 5 categories –

1. Terricolous – lichens growing in soil in hot areas with scanty rain and dry summer. E.g. – *Lecidea*, *Cladonia*.
2. Saxicolous – lichens growing on stones/rocks in cold areas and initiate soil formation. Eg. – *Dermatocarpon*.
3. Arboricolous or Corticolous – growing on bark of trees in tropical areas. Eg- *Usnea*, *Parmelia*.
4. Lignicolous – growing on wood directly. Eg. – *Cyphelium*.
5. Marine aquatic. Eg. – *Hymenelia*, *Caloplaca* – grow on siliceous rocks in sea shores/fresh water bodies.

Nature of lichens

Various views have put forth to explain the physiological nature of association between algal and fungal partner in lichen thallus.

1. Parasitism – holds that fungus parasitizes the algal cells and also lives saprobically on those algal cells which die due to parasitism. This parasitism is of mild type as fungal partner allows most of the algal cells to grow and live. First shown by Schwendener (1867).

It is supported by the following facts – (i) when two components of the lichen thallus are separated, the algal partner is able to live while fungal partner fails to grow independently and die; (ii) fungal partner produces haustoria or appressoria into algal cells to get food and (iii) algal partner does not form its own pectic covering.

2. Symbiosis (Consortium or Mutualism) – (shown by Reinke and de Barry) Fungal and algal partner of lichen thallus have a long healthy life. In this association both partners have mutual growth and derive benefit from each other. Strong evidence in support – When two components of lichen thallus are grown separately in axenic culture and then placed together again, lichenization occurs. Both partners live as husband and wife. The fungal partner has the following functions – (i) it forms structural covering for protection from high light intensity. (ii) fungal hyphae are gelatinous

and absorb water from the environment and transfer it to algal cells. (iii) it also provides some mineral salts to algal partner. (iv) It provides anchorage by its hyphae. (v) It is responsible for reproduction. (vi) It protects alga from dessication by holding water. (vii) It forms major part (up to 99%) of the thallus. In turn, fungal partner takes, by diffusion, carbohydrate (mannitol) synthesized by algal cells and (in case of blue-green algae) it provides nitrogen as well.

Autoradiography studies using C^{14} support this symbiotic relationship.

3. Helotism – Crombie described this association as unnatural between captive algal damsel (slave) and a tyrant fungal master i.e. association decidedly at the cost of alga. The fungal partner is a controlled parasite over the alga. The fungal partner is dominant and forms structural covering while algal partner lives as subordinate or prisoner. Thus, it is a master-slave relationship and it is beneficial slavery for the alga.

Classification

There is no natural system of classification of lichens. On the basis of the structure of fruiting bodies of fungal components, lichens are classified into two groups on the basis of their fungal components (Zahlbruckner, 1907) –

1. Ascolichens – The fungal component of these is a member of the class Ascomycetes. These lichens are divided into two series on the basis of the structure of fruiting bodies –
 - a. Gymnocarpeae/Discolichens – The fruiting body is a disc like apothecium. Eg. – *Parmelia*.
 - b. Pyrenocarpeae/Pyrenolichens – The fruiting body is a flask shaped perithecium. Eg. – *Dermatocarpon*.
2. Basidiolichens – The fungal component of these lichens is a member of the class Basidiomycetes. Eg. – *Corella*, *Dictyonema*.

Structure

The plant body of lichen is thalloid and is irregular in shape. The color of lichens may be green, white, orange, yellow, brown or black but majority are steel grey.

External Morphology

3 types of lichens on the basis of external morphology –

Crustose lichens –

- Encrusting lichens, with an inconspicuous, thin and flat thallus, firm in texture.
- Thallus very closely adhered to substratum and provides a crust-like appearance.
- Wholly or partially embedded in substratum and in the latter case only fruiting bodies visible.
- Eg. – *Graphis*, *Haematomma*, *Rhizocarpon*, *Strigula*, etc.

Foliose lichens –

- Flat with leaf-like and lobed thallus.
- Attached to the substratum with the help of rhizoid-like rhizines.
- Eg. – *Parmelia*, *Peltigera*, etc.

Fruticose lichens –

- Are shrubby lichens with a well developed, shrub-like, cylindrical and branched thallus.
- Grow erect or hang from the substratum.
- Plant body attached to the substratum with the help of a basal mucilaginous disc.
- Eg. – *Alectonia*, *Gladonia*, *Usnea*, etc.

Internal Structure

Internal structure of lichens is very complex. The thallus is composed of algal and fungal components. Such a type of thallus is known as **consortium**. In an advanced foliose lichen, the following 4 distinct regions are recognised in a vertical section –

1. Upper cortex – Outermost thick and protective zone of the thallus; composed of compactly interwoven fungal hyphae arranged at right angle to surface of thallus. No intercellular spaces between the hyphae. In some lichens (eg. – *Parmelia*) there are many irregularly arranged breathing pores on the outer surface. These pores help in gaseous exchange.
2. Algal layer (or Gonidial layer) – occurs just below the upper cortex. The algal cells remain embedded in between the tangled network of loosely interwoven hyphae.

In some species (eg. – *Collema*), algal cells and fungal hyphae are distributed more or less uniformly throughout the thallus. Such species – called **homoisomorous**. On the other hand, in **heteromorous** – the algal cells form a distinct layer within the thallus (eg. – *Parmelia*, etc).

Some common green and blue-green algae found in the lichens are the species of *Protococcus*, *Pleurococcus*, *Cladophora* (Chlorophyceae) and *Nostoc*, *Gleocapsa* and *Rivularia* (Myxophyceae).

3. Medulla – the central part of the thallus; comprising of loosely interwoven fungal hyphae with large spaces between them.
4. Lower cortex – composed of compactly arranged hyphae which run parallel or perpendicular to the surface of the thallus. Some of these hyphae become specialised and help in the attachment of thallus to substratum – called **rhizines**.

Internal structure of crustose lichens is more or less similar to foliose lichens. The lower cortex does not occur in fruiticose lichens due to their cylindrical structure and medulla forms the central part of the axis.

Nutrition

Lichens grow at a very slow rate and hence require a very small amount of food to grow. They can tolerate drought conditions of about 50 weeks because medulla in the thallus acts as water reservoir. They can absorb water from environment. The phycobiont supplies carbohydrates and nitrogen (in case of blue-green algae) to the mycobiont. The phycobiont takes water and minerals from mycobiont. The fungal hyphae are in intimate contact with the algal cells by means of appressoria or haustoria and that various molecules are transferred through the membrane. Fungus partner secretes certain enzymes that make the algal cell wall permeable and thus sugars and other substances are released and taken by fungal hyphae and stored as mannitol in medulla. The fungal hyphae may consume the algal cells died in due course of time.

Reproduction

Lichens reproduce both by asexual and sexual means.

Asexual reproduction

1. Fragmentation – Small fragments of thallus are formed by accidental breaking or by the death or decay of the older parts. Each fragment develops into a new thallus, provided it contains both algal and fungal components.
2. Soredium – Some small bud-like outgrowths, known as soredia, develop on the surface of the thallus. A soredium contains one or few algal cells closely enveloped by a weft of fungal hyphae. The soredia are detached from the thallus by the impact of wind or rain. The soredia germinate on suitable substratum and form new thalli.
3. Cephalodium – are small wart-like structures formed on the surface of the thallus. One of the characteristic features of the cephalodium is that its algal and fungal components differ from that of the thallus due to the fact that cephalodia develop on the younger parts of the thallus from soredia of some other species. Hence, the cephalodium may be regarded as sterile thallus of some other lichen.
4. Isidium – Isidia are small, stalked, greyish-black coral-like outgrowths which develop on the upper surface of the thallus. The isidium has an outer cortical layer enclosing the algal and fungal components. It is usually constricted at the base and is easily detachable from the parent thallus. It germinates under favourable conditions and forms new thallus.
5. Some lichens (eg. – *Physcia*, *Buellia*) develop flask-shaped pycnidia which form pycnidiospores which form fungal hypha on germination and develop into a new lichen on coming in contact with a suitable alga.
6. Sometimes fungal hyphae break to form oidiospores.

Differences between Isidia and Soredia

Isidium	Soredium
It is smaller, column-like.	It is minute, rounded.
It is stalked, corticated, undetachable, black colored.	It is stalkless, noncorticated, detachable, greyish-green in color.
It has many algal cells.	Contains one or a few algal cells.
It is mainly responsible for increasing the photosynthetic area.	It is mainly for vegetative reproduction.

Sexual reproduction

In lichens, the process of sexual reproduction is performed only by the fungal component. Sexual reproduction is similar to that of ascomycetous fungi.

Female sex organ – **Carpogonia** – is differentiated into a basal coiled ascogonium (embedded within algal layer) and an elongated multicellular trichogyne (projects over the surface of thallus).

Male sex organ – **Spermagonia** – is flask-shaped and forms spermatia which function as male gametes.

The spermogonium develops close to carpogonium, enabling spermatia to adhere to the projected part of sticky trichogyne. On dissolution of the walls between the spermatium and trichogyne, the nucleus of spermatium migrates into the carpogonium through trichogyne. The male nucleus fuses with the female nucleus.

Several branched ascogenous hyphae develop from the base of the fertilized ascogonium. The terminal or penultimate binucleate cell of the ascogenous hypha develops into an ascus. The two nuclei fuse to form a diploid nucleus which divides meiotically and mitotically to form eight haploid daughter nuclei. Each haploid metamorphoses into an ascospore.

The asci remain enveloped by paraphysis. The somatic tissue surrounding the asci and paraphysis form the fruiting body, which may be **apothecium** (eg – *Parmelia*, *Aneptychia*) or **perithecium** (eg– *Dermatocarpon*, *Verrucaria*).

The ascospores produce a hypha on germination which, on coming in contact with a suitable alga, forms a lichen thallus.

Comparisons

Lichens and Fungi

In lichens fungal partner is dominant, forming up to 99% of thallus. Only the mycobiont part reproduces sexually. Both lichens and fungi are able to resist extreme cold, hot and dessication. Thus lichens are close to fungi.

But when we culture the fungus in laboratory, it has a very different morphology from that of the lichen thallus from which it has been isolated.

Lichens differ from fungi in form, habit and their physiology.

LICHENS	FUNGI
1 Common in cold climate	1 Thrive best in warm moist climate
2 Tough and leathery in texture	2 Delicate in texture
3 Grow well on barren rocks, stones, tree trunks, sea shores, leaves, walls and in drought conditions.	3 Grow on decaying organic matter living organism and require moisture
4 They grow as epiphytes, lithophytes or terrestrial and are autotrophs	4 They grow as parasites or saprophytes and are heterotrophs.
5 They are composite plants of dual nature.	5 They are simple organisms
6 Reproduce by methods of ascomycetes and basidiomycetes.	6 Reproduce by all fungi methods.
7 Thallus is colored due to organic acids	7 Thallus is usually colorless.
8 Are sun loving and require ample light	8 Are shade loving
9 Grow well in pollution free air	9 Prefer to grow in polluted air.
10 Rate of growth is very slow	10 Rate of growth is very fast.

Economic importance

Lichens are useful not only when they are alive but also when dead.

1. **Ecological Role of Lichens** – Lichens are slow growing but efficient soil makers. They are ecological pioneer of xerarch succession on bare rocks or cooled volcano lava. They are the first plants to grow on dry barren rocks. They secrete carbonic acid which erode rocks and initiate soil formation for other communities (mosses and grasses) to appear. Thus they are called farmers of nature and pioneer colonizers of barren rocks. They are indicators of pure climate. They absorb large amount of CO₂ and liberate equal amount of oxygen. They are very sensitive to SO₂ and polluted air. They do

not grow in heavy polluted area. Their population increases gradually with distance from the polluted area and thus is a measure of pollution intensity. They can tolerate high temperature up to 434° F and cold temperatures up to - 273° C, complete vacuum for six years but cannot tolerate SO₂ and hydrocarbon rich air. SO₂ causes reduction in thickness of thallus, plasmolysis of algal cells, degradation of chlorophyll and failure of soredia formation. SO₂ actually converts chlorophyll of alga into pheophytin by removing MG⁺⁺ from chlorophyll.

SO₂ + moisture in algal cells → H₂SO₃

H₂SO₃ → H⁺ + HSO₃⁻

Chlorophyll + 2H⁺ → Pheophytin + Mg⁺⁺

This pheophytin results in chlorosis and finally death of lichens.

Lichens absorb radioactivity and, therefore, are good to make air pure. Some lichens act as pollution sink and absorb heavy metals like Ni, Co, Mg etc.

2. As Food and Fodder – Lichens are used as food since ancient times. They are important constituents of food in north Polar tundra and eastern Siberian regions. Species of *Lecanora*, *Parmelia*, *Umbilicaria*, and *Cetraria icelandica* (Iceland moss) are some of the lichens which are used as food in many parts of the world. *Umbilicaria esculenta* is a delicacy in Japan, while the species of *Parmelia* are used as curry powder in India.

Lichens contain a polysaccharide – lichenin – but lack true starch and cellulose. *Evernia prunastri* is used by Egyptians for making breads. In France, some lichens are used for making delicious chocolates and pastries. In Japan, *Endocarpon miniatum* is used as a vegetable.

Several lichens (e.g. – *Aspicilia calcarea*, *Lecanora*) are used as food by mites, snails, caterpillars, slugs, termites, etc.

Lichens, such as *Lobaria pulmonaria*, *Evernia prunastri*, are used as fodder for animals. They possess great nutritive value due to the presence of lichenin. *Cladonia rangifera* (reindeer moss) serves as a common food in Tundra regions for animals, specially reindeer and musk ox. Dried lichens are fed to horses and swans.

3. As Medicine – The medicinal value of lichens is due to the presence of lichenin or some astringent substances. Some lichens are used in the treatment of bile, diarrhoea, fever, nervous disorders, hydrophobia and skin diseases. *Parmelia perlata* is specially useful in dyspepsia and in the treatment of snake and scorpion bites. Species of *Usnea* are used to stop bleeding. Usnic acid, obtained from *Usnea* and *Cladonia*, is a broad spectrum antibiotic used in the treatment of infections. Lichens are important constituents of several important medicines. Some lichens are used with tobacco because of their hallucinogenic effects.
4. As Dyes – Dyes obtained from lichens have been used since ancient times for coloring fabrics, etc. Red and purple dyes are obtained from *Ochrolechia androgyna* and *O. tartaria*. Orchil, a blue dye obtained from some lichens (e.g. – *Cetraria icelandica*) is used for dyeing woollens. Orcein, a purified and active principle of orchil dye, is used as a stain in histological studies. Litmus, an important acid-base indicator dye in chemical laboratories, is obtained from *Rocella montagnei* and *Lasallia pustulata*.
5. In Tanning Industry – Some lichens (e.g. – *Cetraria icelandica*, *Lobaria pulmonaria*) are used as tanning agent in leather industries.
6. In Cosmetics and Perfumery – Several species of *Evernia* and *Ramalina* are the source of essential oils, used in the manufacture of cosmetic soaps.
Lichens are also used in the manufacture of hawan samagris, dhup and other perfumeries because of their pleasant smell. They are important ingredients of many antiseptic creams as they have tumour-inhibiting and spasmolytic properties.
7. Fermentation and Distillation – In Russia, Sweden and Siberia, liquors are manufactured by fermentation and distillation of some lichens, such as *Cladonia rangiferina* and *Ramalina fraxinea*.
8. As Culture Media – Lichens are important constituents of some culture media used for the culture of fungi and bacteria in laboratories.

NEGATIVE ROLE – Several lichens are harmful to us. They cause a considerable loss due to etching of glass surfaces and marble stones. Some lichens, such as *Letharia vulpina* (wolf moss), are poisonous.

PART – III

PLANT PATHOLOGY

Prescribed syllabus of Plant Pathology

For the UPSC – CSE Main

Modes of infection and dissemination

Molecular basis of infection and disease resistance/defence

Physiology of parasitism

Control measures

Fungal toxins

Modelling and disease forecasting

Plant quarantine

Important crop diseases caused by viruses, bacteria, mycoplasma, fungi and nematodes

For the UPSC – IFoS Main

Mode of infection and dissemination

Molecular basis of infection and disease resistance/defence

Physiology of parasitism

Control measures

Fungal toxins

Important plant diseases caused by viruses, bacteria, mycoplasma, fungi and nematodes

Some Questions on Physiological Plant Pathology (20 Years: IAS Main 1990 – 2009)

1. 1990 (20 Marks – 200 Words): Role of pectin hydrolases and transeliminases in plant disease development.
2. 1990 (20 Marks – 200 Words): Post infection biochemical defense mechanisms in plants.
3. 1993 (20 Marks – 200 Words): Define Biocontrol. Give examples of diseases controlled by this method.
4. 1994 (20 Marks – 200 Words): What are the criteria that must be met to prove that an organism is a pathogen?
5. 1995 (20 Marks – 200 Words): Briefly describe the physiological changes induced in a host plant by a parasite.
6. 1995 (05 Marks – 50 Words): Mycotoxins
7. 2000 (30 Marks – 300 Words): Fungal toxins
8. 2001 (20 Marks – 200 Words): How does a parasite recognize its host? Describe the molecular basis of host parasite relationship.
9. 2003(20 Marks – 200 Words): Describe various types of pathotoxins and their role in plant diseases.
10. 2005(20 Marks – 200 Words): Write a short account of mycotoxins.
11. 2006(20 Marks – 200 Words): Molecular basis of infection
12. 2008(20 Marks – 200 Words): Write a brief account chemical nature and role of Defensins.
13. 2008(20 Marks – 200 Words): List the major active defense responses of plants in response to pathogen attack.
14. 2008(20 Marks – 200 Words): What is the principle evidence implicating jasmonates in the resistance of plants by insect and pathogen attack.
15. 2009 (20 Marks – 200 Words): No question.

Chapter 1: Fundamental concepts of plant pathology

What is plant pathology?

Plant pathology is the study of:

1. The living entities and environmental conditions that cause disease in plants.
2. The mechanism by which these factors produce disease in plants.
3. The interaction between the disease causing agents and diseased plants.
4. The methods of preventing or controlling disease and alleviating the damage it causes.

What is a plant disease?

A plant is healthy when it can carry out its physiological functions to the best of its genetic potential.

Disease in plants is a series of visible and invisible changes due to a persistent factor which is either a pathogenic organism or an adverse environmental factor which leads to unwanted changes in form, function or integrity of the plant. It may lead to partial impairment or death of plant parts or of the entire plant.

With respect to a crop plant, diseases lead to drop in yield which may be moderate or severe depending on the extent of disease development.

Types of plant diseases

There are two main types of plant diseases.

1. **Non-infectious diseases:** Such diseases do not spread from an affected plant to another plant. Such diseases are caused by abiotic factors. They include the excess, deficiency, non-availability, or improper balance of light, air circulation, relative humidity, water, or essential soil elements; unfavourable soil moisture-oxygen relations; extremes in soil acidity or alkalinity; high or low temperatures; pesticide injury; other poisonous chemicals in air or soil; changes in soil grade; girdling of roots; mechanical and electrical agents; and soil compaction.
2. **Infectious diseases:** In such cases, the disease can spread from an affected plant to a healthy plant. Such diseases are caused by infectious plant pathogens. These agents are listed below in the next section.

Causal factors of plant diseases

Plant diseases are caused by two categories of factors:

1. *Plant pathogens*, which are the disease causing organisms;
2. *Unfavourable environmental conditions* like lack or excess of nutrients, moisture and light or the presence of toxic chemicals in air, water and soil.

What is a plant pathogen?

Any organism that can cause a disease in plants is a plant pathogen.

Organisms which cause infectious diseases in plants are:

1. Viruses
2. Viroids and virus like organisms
3. Bacteria

4. Phytoplasmas
5. Protozoa
6. Fungi
7. Oomycetes
8. Nematodes

In this category we do not include insects, mites, vertebrates or other pests that effect plant health by consumption of plant tissues.

Disease Triangle

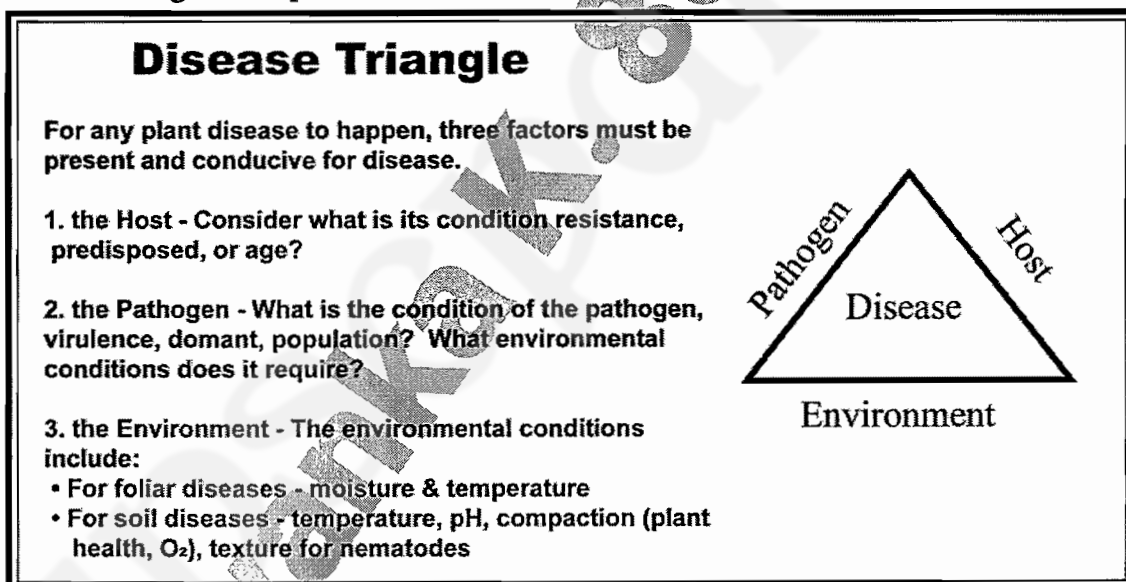
The *Disease Triangle* is a central concept of plant pathology. It is based on the principle that disease is the result of an interaction between a host, a potential pathogen, and the environment. If any one of these factors is missing then disease will not occur.

Thus, there are three fundamental components of disease.

1. Host
2. Pathogen: Virulent and Aggressive
3. Environment

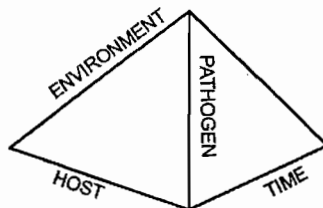
These three components make a disease triangle and show how these three components are interrelated. All the three components are necessary pre- and post- infection.

Disease triangle concept: An illustration



However, some scientists Stevens (1960); Zedler (1972); Van der Plank (1975) have included another Fourth component, i.e. Time in this triangle making it into a plant disease pyramid.

PLANT DISEASE PYRAMID



For the cultivated plants another Fifth component i.e. **Humans** is added as they affect disease development in many ways such as: choice of plants, their level of resistance, their number and density.

An understanding of the disease triangle is a necessary requirement for effective management of the diseases.

Geographic distribution of the disease

On the basis of their extent of occurrence and geographic distribution, diseases are classified as follows:

1. **Endemic Disease:** These are natural to one particular location. When a disease is more or less constantly present from year to year in a moderate to severe form in a particular location it is classified as Endemic.
2. **Epidemic Disease:** They occur periodically but in a severe state involving a major area of the crop. It is generally constantly present but takes severe forms occasionally. This is because the environmental conditions favorable for rapid disease development occur only periodically. Thus, in this case environmental conditions are major determining factor.
3. **Sporadic Disease:** These diseases occur at very irregular intervals and locations and in relatively few instances.

Disease cycle

Plant disease cycles represent pathogen biology as a series of interconnected stages of development including dormancy, reproduction, dispersal, and pathogenesis. The progression through these stages is determined by a continuous sequence of interactions among host, pathogen, and environment.

In simple terms, disease cycle is a cyclic sequence of events through which a pathogen attacks a host, exploits it, carries out its own multiplication; causes disease symptoms and spreads from a diseased host to a healthy host.

The **seven main events** of a plant disease cycle include the following. (Details in Chapter 3)

1. Inoculation
2. Pre – Penetration
3. Penetration
4. Infection
5. Growth and Reproduction of Pathogen
6. Dissemination of the Pathogen
7. Seasonal carryover of the Pathogen.

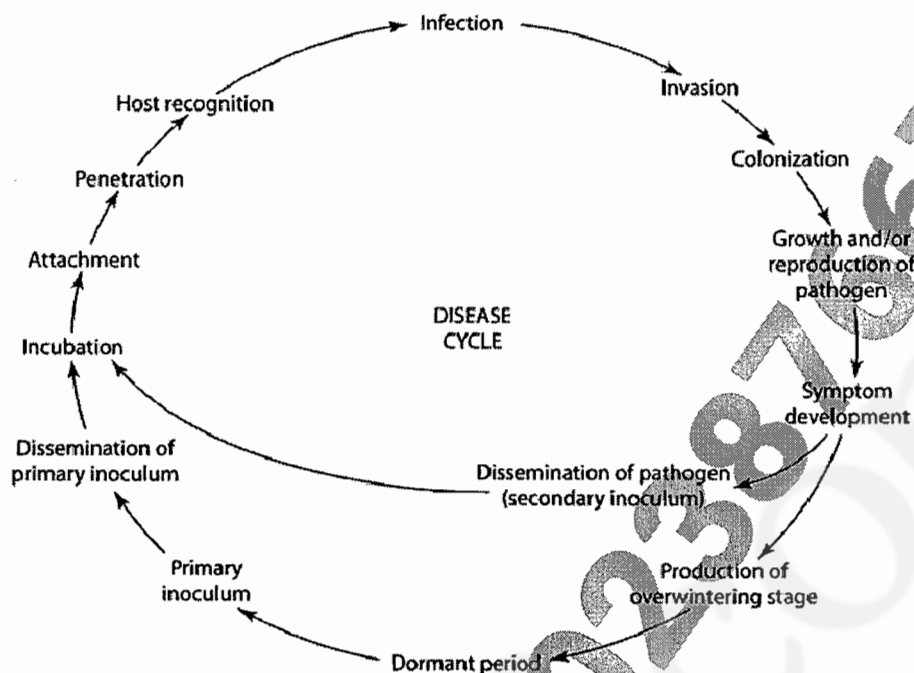


Figure: Stages in development of a disease cycle

Symptoms of common plant diseases

Symptoms are the internal and external expressions of disease.

Generalized symptoms may be classified as local or systemic, primary or secondary, and microscopic or macroscopic.

- **Local symptoms** are physiological or structural changes within a limited area of host tissue, such as leaf spots, galls, and cankers.
- **Systemic symptoms** are those involving the reaction of a greater part or all of the plant, such as wilting, yellowing, and dwarfing.
- **Primary symptoms** are the direct result of pathogen activity on invaded tissues (e.g., swollen "clubs" in clubroot of cabbage and "galls" formed by feeding of the root-knot nematode).
- **Secondary symptoms** result from the physiological effects of disease on distant tissues and uninvaded organs (e.g., wilting and drooping of cabbage leaves in hot weather resulting from clubroot or root knot).
- **Microscopic disease symptoms** are expressions of disease in cell structure or cell arrangement seen under a microscope.
- **Macroscopic symptoms** are expressions of disease that can be seen with the unaided eye.

Specific macroscopic symptoms are classified under four major categories:

1. Prenecrotic
2. Necrotic
3. Hypoplastic
4. Hyperplastic or hypertrophic

These categories reflect abnormal effects on host cells, tissues, and organs that can be seen without a hand lens or microscope.

Table: Plant disease symptoms

	Description and causes	Examples
Prenecrotic	Symptom expression that precedes the death of cells or the disintegration of tissues	
Water-soaking	A water-soaked, translucent condition of tissues caused by water	Late blight lesions on potato and tomato

	moving from host cells into intercellular spaces	leaves; bacterial soft rot of fleshy vegetables
Wilting	Temporary or permanent drooping of leaves, shoots, or entire plants from lack of water	Bacterial wilt of cucumber; <i>Fusarium</i> wilt of tomato
Abnormal coloration	Yellowing, reddening, bronzing, or purpling in localized areas of leaves where chlorophyll has been destroyed; may be due to a variety of causes	Cabbage and aster yellows; halo blight of beans; potassium or phosphorus deficiency
	The presence of two or more colours in leaves and flowers due to a genetic abnormality is called variegation; viral infection results in "flower breaking"	Tulip mosaic
Necrotic	Localized or general death of cells or disintegration of tissues	
Blast	Sudden blighting or death of young buds, flowers, or young fruit; failure to produce fruit or seeds	<i>Botrytis</i> blight of peony buds; oat blast
Bleeding	Flow of sap, often discoloured, from a split crotch, branch stub, or other wound; usually accompanied by an odour of fermentation	Bleeding canker of beech, dogwood, and maple
Blight	Sudden or total discoloration and killing of large numbers of blossoms, leaves, shoots, or limbs or the entire plant; usually young tissues are attacked; the disease name is often coupled with the name of the host and the part attacked—blossom blight, twig blight, tip blight	Fire blight of pome fruits; <i>Diplodia</i> or <i>Sphaeropsis</i> tip blight of conifers
Canker	A definite, dead, often sunken or swollen and cracked area on a stem, limb, trunk, tuber, or root surrounded by living tissues	Anthrax of sycamore and brambles; <i>Nectria</i> canker of hardwoods; fire blight of pome fruits
Damping-off	Decay of seed in soil, rapid death of germinating seedlings before emergence, or emerged seedlings suddenly wilting, toppling over, and dying from rot at or near the soil line	Preemergence damping-off and postemergence damping-off; both are common in seedbeds
Dieback	Progressive browning and death of shoots, branches, and roots starting at the tips	Winter injury; wet soil; excess soil nutrients; girdling cankers; stem or root rots; nematodes
Firing	Drying and dying of leaves	Nitrogen or potassium deficiency in corn; <i>Verticillium</i> wilt of eggplant
Fleck	A small, white to translucent spot or lesion visible through a leaf	Ozone injury to many plants; necrotic fleck of lily
Mummification	Final stage in certain fruit rots, in which the dried, shriveled, and wrinkled fruit is called a "mummy"	Brown rot of stone fruits; black rot of apple
Net necrosis	An irregular crisscrossing of dark brown to black lines giving a netted appearance	In potato tubers of plants with virus leaf roll
Pitting	Small dead areas within fleshy or woody tissue that appears healthy externally; definite sunken grooves or pits are formed	Virus stem-pitting in apple and peach trunks; stony pit of pear fruit
Rot	Decomposition and putrefaction of cells, later of tissues and organs; the rot may be dry, firm, watery, or mushy and is characterized by such names as hard rot, soft rot, dry rot, black rot, and white rot	Bacterial soft rot; berry rot; bud rot; bulb rot
Scald	Blanching of young fruit, foliage, and shoot tissue; generally superficial	Sunscald; apple and pear scald
Scorch	Sudden death and "burning" of large, indefinite areas in leaves and	Toxicity from pesticides and air pollutants; drought; wind; lack or excess

	fruit	of some nutrient
Shot hole	Dead spotting of leaves with diseased tissue dropping out, leaving small holes	Bacterial spot; <i>Coryneum</i> blight of peach
Spot	A definite, localized, round to regular lesion, often with a border of a different colour, characterized as to location (leaf spot, fruit spot) and colour (brown spot, black spot); if numerous or if spots enlarge and merge, a large irregular blotch or blight may develop	Gray leaf spot of tomato; black spot of rose; tar spot of maple
Staghead	An advanced form of dieback applied to a tree in which large branches in the upper crown are killed	Oak wilt on bur oak; dwarf mistletoe on Douglas fir; <i>Armillaria</i> root rot of oak
Streak	Narrow, elongated, somewhat superficial necrotic lesions, with irregular margins, on stems or leaf veins	Virus streak of pea, raspberry, and tomato; Stewart's wilt of sweet corn
Stripe	Narrow, elongated, parallel, necrotic lesions especially in leaf diseases of cereals and grasses	<i>Helminthosporium</i> stripe of barley; <i>Scolecotrichum</i> brown stripe of forage grasses
Hypoplastic	The underdevelopment of plant cells, tissues, or organs	
Abortion	Halting development of an organ after partial differentiation	Ergot of rye and other grasses
Chlorosis	Yellowing or whitening of normal green tissue due to partial or complete failure of chlorophyll to develop	Strawberry and aster yellows; genetic variegation in corn; iron deficiency of azalea
Stunting or dwarfing	The underdevelopment of the plant or some of its organs	Dahlia stunt or mosaic; curly top of beans; little-leaf disease of pines
Rosetting	Shortening of internodes of shoots and branches, producing a bushy growth habit	Peach and lily rosette
Hyperplastic or hypertrophic	An overdevelopment or overgrowth of plant cells, tissues, or organs; hyperplastic has come to mean an increase in number of cells, hypertrophic an increase in cell size	
Abscission or cast	Early dropping of leaves, flowers, or small fruits; usually associated with premature formation of an abscission (separation) cell layer	Black spot of rose; early blight of tomato; apple scab
Callus	Overgrowth of tissues, often at margins of a canker or wound	<i>Nectria</i> canker of hardwoods; stem pitting of peach
Curl	Distortion and crinkling of leaves or shoots resulting from unequal cell growth of opposite sides or in certain tissues	Tobacco and tomato mosaic; leaf roll of potato; peach leaf curl
Epinasty	Downward or outward curling and bending of a leaf or petiole	<i>Fusarium</i> wilt of tomato
Fasciation, or witches'-broom	A distortion that results in a dense, bushy overgrowth of thin, flattened, and sometimes curved shoots, flowers, fruit, and roots at a common point; usually due to adventitious (abnormally located) development of organs	Witches'-broom of hackberry; hairy root of apple; leaf gall or fasciation of geranium (see also <i>Rosetting</i> under <i>Hypoplastic</i> in this table)
Metamorphosis or transformation	Development of more or less normal tissues or organs in an abnormal location	Crazy-top of corn and sorghum; formation of aerial potato tubers
Proliferation	Continued development of an organ after it would normally stop growing	Adventitious shoots in China aster and chrysanthemum from aster yellows mycoplasma
Russetting	Usually a brownish, superficial roughening or corking of the epidermis of leaves, fruit, tubers, or other organs; often due to suberization (cork development) of cells following injury	Spray or weather injury to apples; sweet potato scurf
Scab	Roughened to crustlike, more or less circular, slightly raised or	Apple, peach, and cucumber scab;

	sunken lesions on the surface of leaves, stems, fruit, or tubers	common scab of potato
Gall, knot, or tumefaction	Formation of local, fleshy to woody outgrowths or swellings; the outgrowth is often composed of unorganized cells	Crown gall; black knot of plum; <i>Fusiform</i> gall rust of pine; nematode galls

Signs of common plant diseases

Besides symptoms, the diagnostician recognizes signs characteristic of specific diseases. Signs are either structures formed by the pathogen or the result of interaction between pathogen and host—e.g., ooze of fire blight bacteria, slime flux from wetwood of elm, odour of tissues affected with bacterial soft rot.

Table: Signs of pathogen presence in diseased plants

Sign	Description	Examples
Acervulus	A shallow, saucer-shaped fungal structure that bears asexual spores (conidia); it is usually formed below the cuticle or epidermis of leaves, stems, and fruits, later rupturing the surface and exposing its spore-bearing surface	Anthrachnose of muskmelon and tomato; <i>Marssonina</i> leaf spot and twig blight of poplar
Apothecium	A disk-, saucer-, or cup-shaped fungal structure that produces sexual spores (ascospores); it is often stalked and fleshy	Brown rot of stone fruits; <i>Sclerotinia</i> white mold of fleshy vegetables
Cleistothecium	A speck-sized, black fruiting body completely enclosing sexual spores	Many powdery mildew fungi
Conidiophores	Asexual fungal structures of various colours that bear conidia and appear powdery, velvety, or downy en masse; they often cover lesions of leaf, stem, or fruit	<i>Botrytis</i> blight or gray mold of many flowers; <i>Penicillium</i> mold of citrus fruit; downy mildew of grape
Conk or punk	Fruiting body (sporophore) of wood-rotting fungi that produces tremendous numbers of spores (up to 100 billion per day); conks are usually large and woody and are found on tree stumps, branches, or trunks	<i>Fomes</i> and <i>Polyporus</i> wood rots of hardwoods and conifers
Mushrooms (toadstools)	Fleshy, umbrella-shaped fruiting bodies of wood-decay fungi	<i>Armillaria</i> and <i>Clitocybe</i> root rots
Mycelium	The vegetative body of a fungus, which is composed of a mass of branched filaments (hyphae) often interwoven into a feltlike or woolly mass	<i>Rhizopus</i> soft rot of sweet potato and leak of strawberry; <i>Sclerotinia</i> white mold of beans
Nematode cysts	Round to lemon-shaped, speck-sized bodies, white to brown in colour, are diagnostic for cyst nematodes; they are often evident on the root surface	Sugar beet, soybean, and clover cyst nematodes
Odours	The process of host colonization and many pathogens give off characteristic odours	Bacterial soft rot; stinking smut or bunt of wheat; slime flux of elm
Ooze or exudate	Droplets of bacteria or fungal spores, usually mixed with host cell decomposition products, found on surfaces of lesions	Ooze from fire blight; scab on cucumber fruit; cut stem of cucumber affected with bacterial wilt
Perithecium	Speck-sized fungal fruiting body that produces large numbers of sexual spores; perithecia are dark-coloured, round to flask-shaped, usually partially buried in diseased tissue; they resemble pycnidia	Apple and pear scab; <i>Gibberella</i> stalk and ear rot of corn
Powdery mildew	White, powdery to mealy, superficial growths of mycelia and conidiophores on surfaces of leaves, stems, flowers, and fruit	Powdery mildew diseases of bluegrass, phlox, zinnia, and rose (see also <i>Cleistothecium</i> , this table)
Pycnidium	Speck-sized fungal fruiting body that produces large numbers of asexual spores (conidia); pycnidia are dark-coloured, round to flask-shaped, usually partially	<i>Septoria</i> leaf spots; <i>Diplodia</i>

	buried in diseased tissue; they resemble perithecia	stalk rot of corn
Rhizomorphs	Cordlike or rootlike strands, composed of a bundle of closely intertwined hyphae, by which certain fungi make their way through soil and over or under bark of woody plants	<i>Armillaria</i> and <i>Clitocybe</i> root rots; <i>Sclerotium rolfsii</i> stem rot of peanuts
Sclerotium	Brown to black, compact, hard resting body of certain fungi with a rindlike covering; the size varies from a fly speck to a large sweet potato depending on the fungus forming it	Ergot of rye; onion white rot
Seed	Dodder seed is a sign of this parasitic flowering plant when found in clover or alfalfa seed	Dodder (<i>Cuscuta</i> , about 170 species)
Sorus (pustule)	A compact mass of spores, or a cluster of sporangia (spore-bearing structures), produced in or on the host by fungi causing such diseases as white rust, smut, and true rust; before rupturing, the sorus is normally covered by host epidermis	White rust of crucifers; corn and bluegrass smuts; black stem rust of cereals
Spores	Microscopic, usually single- or few-celled reproductive bodies of fungi corresponding in function to seeds of higher plants; spores vary greatly in size, shape, and colour; they are asexually produced or result from sexual processes; asexual spores may be formed directly from vegetative hyphae but often are produced in special fruiting structures (e.g., acervulus, coremium, pycnidium, and sporodochium)	
Sporodochium	A cushion-shaped stroma covered with conidiophores bearing asexual spores; found scattered in leaf, stem, and fruit lesions	<i>Cercospora</i> leaf spot of celery and sugar beet; brown rot of stone fruits; <i>Fusarium</i> blight of bluegrass
Stroma	A crustlike or cushionlike mass of fungal hyphae often intermingled with host tissue on or in which spores are produced—usually in reproductive bodies	Tar spot of maple and sycamore
Synnema or coremium	A tight cluster of erect conidiophores forming an elongated column on which asexual spores are borne	Dutch elm disease; oak wilt; black rot of sweet potato

Chapter 2: The causes of plant diseases

Phytopathology (plant pathology) is the scientific study of plant diseases caused by pathogens (infectious diseases) and environmental conditions (physiological factors). Plant Pathology also involves the study of the identification, etiology, disease cycle, economic impact, epidemiology, pathosystem genetics and management of plant diseases.

According to G.N. Agrios (1997), *Plant Pathology is the study of (1) the living entities and the environmental conditions that cause disease in plants; (2) the mechanisms by which these factors produce disease in plants; (3) the interactions between the disease causing agents and the diseased plant; and (4) the methods of preventing or controlling disease and alleviating the damage it causes.*

Organisms which cause infectious disease are called plant pathogens and they include fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants. The insects, mites, vertebrate or other pests which affect plant health by consumption of plant tissues are not regarded as plant pathogens.

Causes of plant diseases

As already mentioned, plant diseases are caused by:

1. Environmental conditions plus physiological factors
2. Pathogen organisms (including fungi, oomycetes, bacteria, viruses, viroids, virus-like organisms, phytoplasmas, protozoa, nematodes and parasitic plants); and

Environmental Factors Causing Plant Diseases

When environmental factors are the reasons behind plant pathogenesis, the disease becomes known as Physiological Plant Disorder. Physiological plant disorders are caused by *abiotic factors* such as poor light, weather damage, water-logging or a lack of nutrients, and affect the functioning of the plant system.

Causes of physiological disorders can be identified by examining the following issues.

1. Where symptoms first appear on a plant- on new leaves, old leaves or all over?
2. The pattern of any discolouration or yellowing- is it all over, between the veins or around the edges? If only the veins are yellow, then deficiency of nutrients is probably not the cause.
3. Note general patterns rather than examining individual plants- are the symptoms distributed throughout a group of plants of the same type growing together. In the case of a deficiency all of the plants should be similarly affected.
4. Soil analysis, such as determining pH, water condition, mineral composition etc. can help to confirm the presence of physiological disorders. Recent conditions, such as heavy rains, dry spells, frosts, etc, may also help to determine the cause of plant disorders.

Significant physiological disorders can be caused in nature by:

1. Drought
2. Frost damage, and breakage by snow and hail

3. Flooding and poor drainage
4. Nutrient deficiency
5. Salt deposition and other soluble mineral excesses (e.g. gypsum)
6. Wind (windburn, and breakage by hurricanes and tornadoes)

Characterization of a pathogen

To pronounce an organism as a plant pathogen, the plant pathologists rely on **Koch's postulates**.

Koch's postulates are **four criteria** designed to establish a causal relationship between a microbe and a disease. The postulates were formulated by Robert Koch and Friedrich Loeffler in 1884 and refined and published by Koch in 1890. Koch applied the postulates to establish the etiology of Anthrax and Tuberculosis, but they have been generalized to other diseases.

These four criteria must be satisfied before a microorganism isolated from a diseased human, animal, or plant can be considered as the cause of the disease.

The four criteria are:

1. Pathogen must **ALWAYS** be associated with disease in **ALL** diseased plants. There are no exceptions allowed.
2. Pathogen must be isolated and established in **PURE** culture. This may be difficult with obligate parasites, but methodologies have been developed to fulfill this requirement even with obligate parasites.
3. Inoculation of a healthy plant of the same variety must reproduce **EXACTLY** the same symptom(s). Inoculation must be of a healthy plant of the same species and cultivar. This may be difficult if one isolates from a plant of unknown cultivar. The symptoms must be reproduced essentially identical to the initial diseased plant, taking into account differences between the initial plants environment and the healthy inoculated plant.
4. Pathogen must be re-isolated from inoculated plant and its identity confirmed as the same as the original isolate. The organism recovered must be the identical to the original isolate. There are no exceptions.

In effect, Koch's Postulates are the scientific method applied to Pathobiology. Without them, Pathobiology becomes an art and not a science.

The major classes of plant pathogens

Organisms that cause infectious disease include:

1. Fungi and Oomycetes
2. Bacteria
3. Viruses
4. Viroids and Virus-like organisms
5. Phytoplasmas (Mollicutes)
6. Protozoa
7. Nematodes
8. Parasitic plants

Not included are ectoparasites like insects, mites, vertebrate, or other pests that affect plant health by consumption of plant tissues.

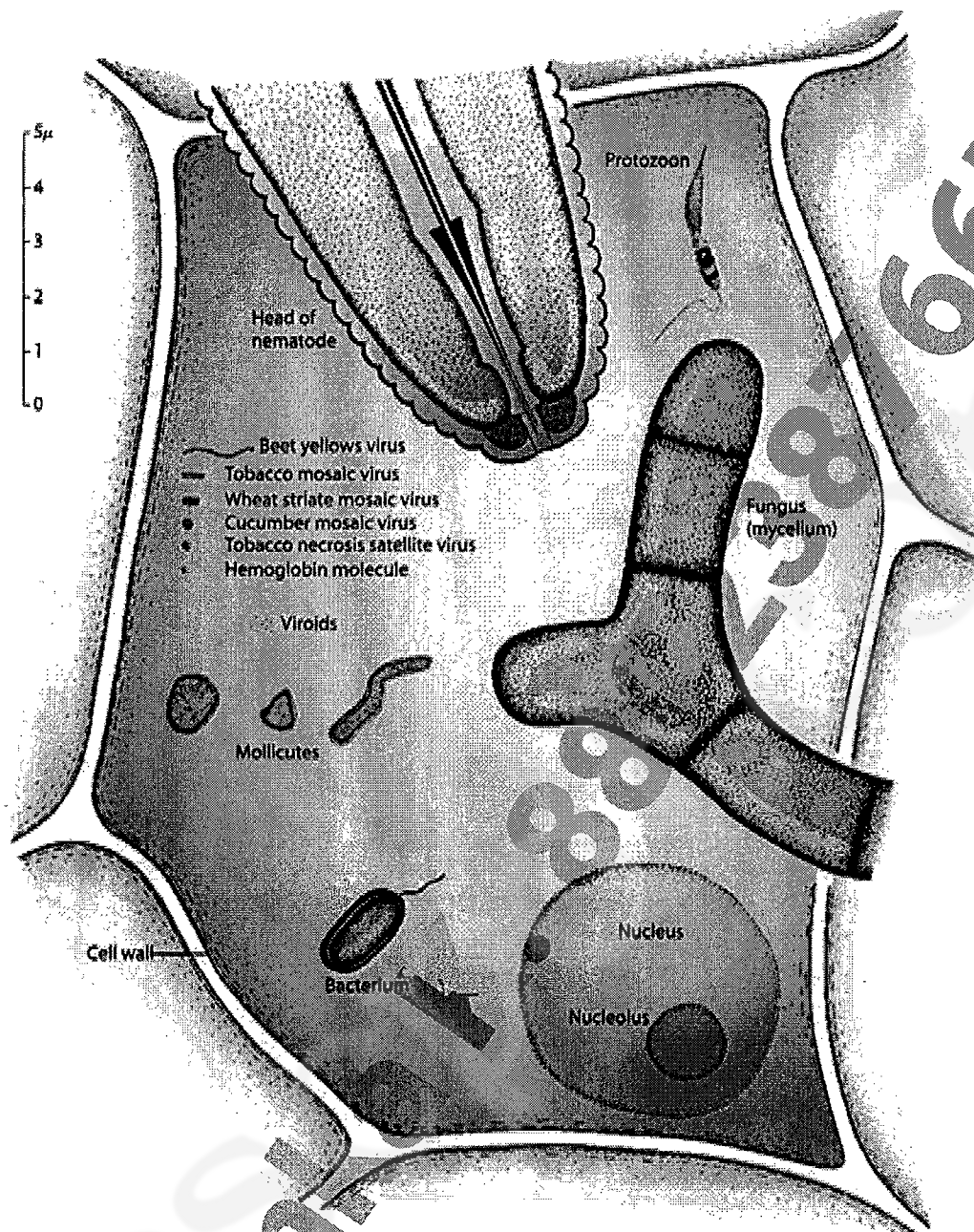


FIGURE Schematic diagram of the shapes and sizes of certain plant pathogens in relation to a plant cell. Bacteria, mollicutes, and protozoa are not found in nucleated living plant cells.

The major classes of plant pathogens are described below.

Fungi and Fungi – like organisms

While plant diseases may be caused by environmental factors, viruses, mycoplasma, bacteria, nematodes, a few protozoa, and parasitic higher plants, the majority - more than 75% - of plant diseases are caused by fungi and fungi like organisms. All of the major groups of fungi have species which are plant parasitic.

1. **Plasmodiophoromycetes** (currently in the Kingdom Protoctista): This class contains endophytic slime molds, because they produce amoeboid cells and plasmodia within the cells of their host. The most

important of parasites are *Plasmodiophora brassicae* that causes club root of cabbage and related plants, and *Spongopora subterranean*, the cause of powdery scab of potatoes.

2. **Chytridiomycota:** Several different groups of chytrids parasitize plants. *Oplidium* spp. infect pollen, algae, other fungi, and several different groups of higher plants. *Physoderma maydis* causes brown spot or streak of corn leaves and species of *Synchytrium* cause wart of potatoes. In cool, moist climates, chytrids can cause extensive damage to crop plants.
3. **Oomycetes** (currently in the Kingdom Stamenopila): There are four orders of Oomycetes, all characterized by producing heterokont zoospores, (with one whiplash and one tinsel flagellum) asexually and oospores sexually. The most economically important group of Oomycetes is the Peronosporales that contain the late blight of potato fungus *Phytophthora infestans* and relatives such as *Peronospora*, *Bremia*, *Plasmopara* and others that cause "downy mildews", the "damping off" fungi, *Pythium* spp., and the white rust fungi, *Albugo* spp.
4. **Zygomycota:** The most important order of Zygomycetes that cause diseases of plants and decay of plant products is the Mucorales. Members of the bread mold genus *Rhizopus* causes soft rots of vegetables and fruits. Species of a related fungus, *Choanephora*, causes blossom blight and decay of squash and similar vegetables.
5. **Ascomycota:** This is one of the largest and most complex groups of fungi. There are various groups of Ascomycetes recognized by the way they produce their asci.

Archiascomycetes & Hemiascomycetes: Yeasts such as *Saccharomyces* and others are very important in the spoilage of grains, fruits, and vegetables. Species of *Taphrina* causes swelling and distortion of leaves, flowers, and fruit of a number of plants. An important one is *Taphrina deformans* that causes peach leaf curl. Others cause oak leaf blister and similar foliar diseases. *Nematospora* causes seed decay and root rot on plants such as cotton.

Plectomycetes: The most important plant parasitic genus in the Plectomycetes is *Ophiostoma* (*Ceratocystis*). *O. ulmi* is the cause of the Dutch elm disease.

Pyrenomycetes: An important pathogenic group is the Erysiphales, or powdery mildews, that grow on the epidermis of leaves, forming asexual spores in abundance, thus, powdery mildew. The common genera of powdery mildews include: *Erysiphe* which is common on grasses, *Phyllactinia* on oaks and other trees, and *Uncinula* on grapes and other shrubs.

Several perithecial ascomycetous fungi are important plant parasites, including species of *Nectria*, *Fusarium*, *Verticillium*, and *Gliocladium*. They cause wilts, cankers, root rots, and a variety of plant problems. The Clavicipitales are important plant parasites. *Claviceps purpurea* occurs on wheat, rye, barley, and a number of cereal grasses. While their overall damage to grasses is not extensive, the sclerotia (ergots) are of great concern because of their toxic effects on man and other animals (ergotism).

Discomycetes are the cup-fungi and their allies. Important plant pathogens in this group include *Rhytisma*, the cause of tar spot of maple, *Sclerotinia* which causes various stromatic rots of vegetables, and *Monilinia*, the cause of brown rot of peaches and similar fruits.

Loculoascomycetes: This is a large groups of stromatic Ascomycetes in which the bitunicate asci are borne within stroma and lyse a cavity. From one to six orders have been recognized in this group, all have plant parasitic members. Among the Myriangiales there is citrus scab caused by species of *Elsinoe*. In the Dothideales there are species of *Capnodium* that cause sooty molds of plants, *Mycosphaerella* that cause leaf spots of plants, and one that causes greasy spot of citrus. Among the Pleosporales, *Venturia inaequalis*, the cause of apple scab. Species of *Gaeumannomyces* cause takeall disease of wheat.

6. **Deuteromycetes:** This is an artificial group of fungi that is made up of the conidial (asexual) states of various fungi, but largely Ascomycetes. Species of *Alternaria*, *Bipolaris*, *Botrytis*, *Cercospora*, *Diplodia*,

Dreschlera, *Exerohilum*, *Fusarium*, *Phoma*, *Phomopsis*, *Rhizoctonia*, and *Verticillium* are among the most common groups that cause molds, blights, cankers, leaf spots, root rots and other maladies.

- Basidiomycetes:** Among plant diseases caused by Basidiomycetes we find the rusts, smuts, felt fungi, root rots, heart rots, and thread-blights. The Uredinales represent the rust fungi. The most important rust fungi include *Puccinia graminis* with many varieties that attack wheat, oats, rye, barley etc. Other important rusts include *Puccinia arachidis* on peanuts, species of *Cronartium* that causes rusts of pines, with an inconspicuous stage on many of our oak species; *Phragmidium*, the rust of roses, and *Melampsora* on flax, beans and other hosts, and species of *Gymnosporangium* that causes cedars-apple rust in which the fungus alternates between cedar and apple trees.

The Ustilaginales encompass the smut fungi. Their spores are formed inside of blister like pustules on leaves, but more commonly on inflorescences of plants. When spores mature in these blister-like pustules they give a smutty appearance. Species of *Ustilago* are common rusts on corn, wheat, rye and barley.

Tilletia causes bunt and stinking smut of wheat. The Exobasidiales are a small group of mainly leaf parasites that occur mostly on Ericaceous plants, i.e. the blueberry family. They cause galls very similar to the galls that are formed by *Taphrina* in the peach leaf curl.

The mushroom order Agaricales is largely mycorrhizal or saprobic, but a few are clearly parasitic. This includes species of *Armillaria* that causes root rots on a number of trees and ornamental species.

The Bacteria

Most bacteria that are associated with plants are actually saprophytic, and do no harm to the plant itself. However, a small number, around 100 species, are able to cause disease. Bacterial diseases are much more prevalent in sub-tropical and tropical regions of the world. Significant bacterial plant pathogens include Proteobacteria like *Xanthomonas* spp. and *Pseudomonas* spp.

Most plant pathogenic bacteria are rod shaped (bacilli). In order to be able to colonise the plant they have specific pathogenicity factors. There are 4 main bacterial pathogenicity factors:

- Cell wall degrading enzymes - used to break down the plant cell wall in order to release the nutrients inside. Used by pathogens such as *Erwinia* to cause soft rot.
- Toxins These can be non-host specific, and damage all plants, or host specific and only cause damage on a host plant.
- Phytohormones - for example *Agrobacterium* changes the level of Auxin to cause tumours.
- Exopolysaccharides - these are produced by bacteria and block xylem vessels, often leading to the death of the plant.

Bacteria control the production of pathogenicity factors via quorum sensing.

Phytoplasmas ('Mycoplasma-like organisms') and spiroplasmas

Phytoplasma and *Spiroplasma* are prokaryotes which lack cell walls, and are related to the mycoplasmas which are human pathogens. Together they are referred to as the mollicutes. They also tend to have smaller genomes than true bacteria. They are normally transmitted by sap-sucking insects, being transferred into the plants phloem where it reproduces. They cause Yellowing diseases in various plants.

Viruses, viroids and virus-like organisms

There are many types of plant virus, and some are even asymptomatic. Normally plant viruses only cause a loss of yield. Therefore it is not economically viable to try to control them, the exception being when they infect perennial species, such as fruit trees.

Most plant viruses have small, single stranded RNA genomes. These genomes may only encode 3 or 4 proteins: a replicase, a coat protein, a movement protein to allow cell to cell movement and sometimes a protein that allows transmission by a vector.

Plant viruses must be transmitted from plant to plant by a vector. This is normally an insect, but some fungi, nematodes and protozoa have been shown to be viral vectors.

Nematodes

Nematodes are small, multicellular wormlike creatures. Many live freely in the soil, but there are some species which parasitize plant roots. They are mostly a problem in tropical and subtropical regions of the world, where they may infect crops. Root knot nematodes have quite a large host range, whereas cyst nematodes tend to only be able to infect a few species. Nematodes are able to cause radical changes in root cells in order to facilitate their lifestyle.

Protozoa

There are a few examples of plant diseases caused by protozoa. They are transmitted as zoospores which are very durable, and may be able to survive in a resting state in the soil for many years. They have also been shown to transmit plant viruses.

When the motile zoospores come into contact with a root hair they produce a plasmodium and invade the roots.

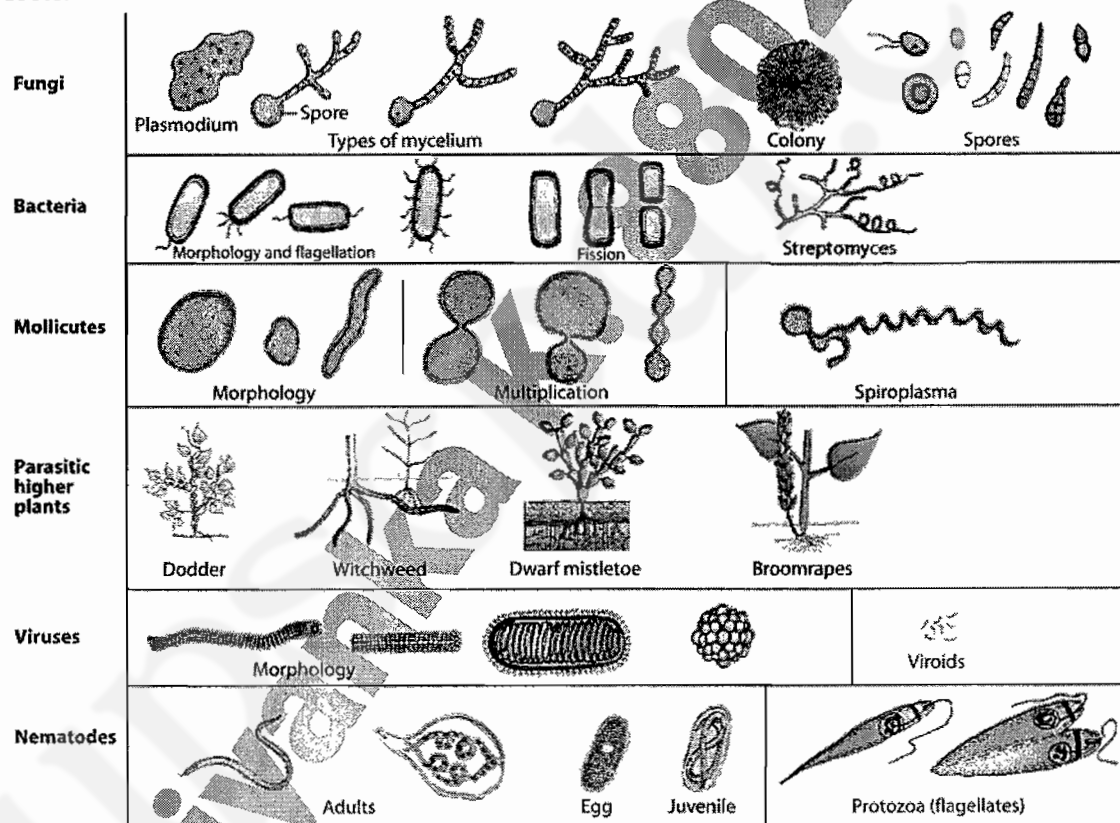
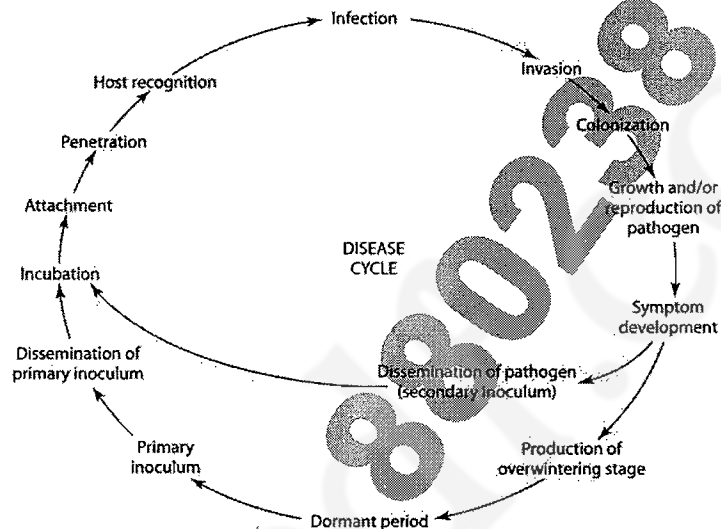


Figure: Morphology and ways of multiplication of some of the groups of plant pathogens

Chapter 3: Modes of infection and dissemination

Modes of infection

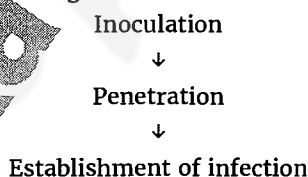
Infection is the process by which pathogens establish contact with susceptible cells or tissues of the host and procure nutrients from them. Following infection, pathogens grow, multiply, or both within the plant tissues and invade and colonize the plant to a lesser or greater extent. Growth and/or reproduction of the pathogen (colonization) in or on infected tissues are actually two concurrent substages of disease development as shown below.



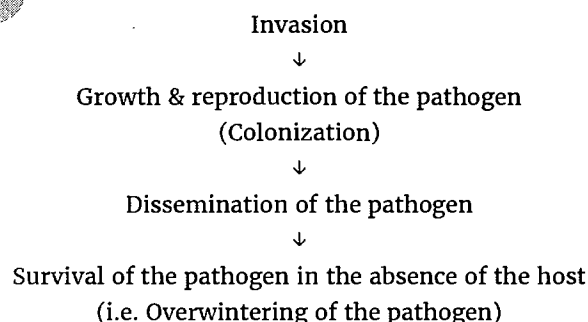
There is a significant variation in the modes of infection in plant diseases, depending on the pathogen and the host involved. A brief overview is provided below.

Steps leading to infection

As shown in the figure above, the steps leading to infection are as follows.



The events taking place after successful infection are as follows.



Inoculation

Inoculation is the initial contact of a pathogen with a site of plant where infection is possible. The pathogen(s) that lands on or is otherwise brought into contact with the plant is called the **inoculum**.

The inoculum is any part of the pathogen that can initiate infection. Thus, in fungi the inoculum may be spores, sclerotia (i.e., a compact mass of mycelium), or fragments of mycelium. In bacteria, mollicutes, protozoa, viruses, and viroids, the inoculum is always whole individuals of bacteria, mollicutes, protozoa, viruses, and viroids, respectively.

In nematodes, the inoculum may be adult nematodes, nematode juveniles, or eggs.

In parasitic higher plants, the inoculum may be plant fragments or seeds. The inoculum may consist of a single individual of a pathogen, e.g., one spore or one multicellular sclerotium, or of millions of individuals of a pathogen, e.g., bacteria carried in a drop of water. One unit of inoculum of any pathogen is called a **propagule**.

Types of Inoculum

An inoculum that survives dormant in the winter or summer and causes the original infections in the spring or in the autumn is called a **primary inoculum**, and the infections it causes are called primary infections. An inoculum produced from primary infections is called a **secondary inoculum** and it, in turn, causes secondary infections.

Landing or Arrival of Inoculum

The inoculum of most pathogens is carried to host plants passively by wind, water, and insects.

An airborne inoculum usually gets out of the air and onto the plant surface not just by gravity but by being washed out by rain. Only a tiny fraction of the potential inoculum produced actually lands on susceptible host plants; the bulk of the produced inoculum lands on things that cannot become infected.

Some types of inoculum in the soil, e.g., zoospores and nematodes, may be attracted to the host plant by such substances as sugars and amino acids diffusing out of the plant roots.

Vector-transmitted pathogens are usually carried to their host plants with an extremely high efficiency.

Penetration

With respect to penetration, the following modes are observed.

1. **Placement of pathogen inside the cell:** Pathogens such as mollicutes, fastidious bacteria, protozoa, and most viruses are placed directly into cells of plants by their vectors. In most cases, they are surrounded by cytoplasm, cytoplasmic membranes, and cell walls.
2. **Placement of pathogen on the plant surface:** Almost all fungi, bacteria, and parasitic higher plants are first brought into contact with the external surface of plant organs. Before they can penetrate and colonize the host, they must first become attached to the host surface. Attachment takes place through the adhesion of spores, bacteria, and seeds through adhesive materials. These adhesive materials vary significantly in composition and in the environmental factors they need to become adhesive. These variations are summarized below.
 - a. **Attachment requiring moisture:** The propagules of some pathogens have on their surface or at their tips mucilaginous substances consisting of mixtures of polysaccharides, glycoproteins, lipids, and fibrillar materials, which, when moistened, become sticky and help the pathogen adhere to the plant. For example, in some fungi, hydration of the spore by moist air or dew causes the extrusion of preformed mucilage at the tip of the spore that serves for the immediate adherence of the spore to the hydrophobic plant surface and resistance to removal by flowing water.
 - b. **Attachment not requiring moisture:** In powdery mildew fungi, which do not require free water for infection, adhesion is accomplished by release from the spore of the enzyme cutinase, which makes the plant and spore areas of attachment more hydrophilic and cements the spore to the plant surface.
 - c. **Attachment requiring synthesis of new material:** In some cases, propagule adhesion requires on-the-spot synthesis of new glycoproteins and it reaches maximum levels in about 30 minutes after contact.
3. **Modes of entry of the pathogens which arrive on plant surface:** With respect to fungal pathogens, which almost always arrive on the plant surface, three modes of penetration are seen.
 - a. Active penetration
 - b. Penetration through natural openings

c. Penetration through wounds

These modes are summarized in the figure below.

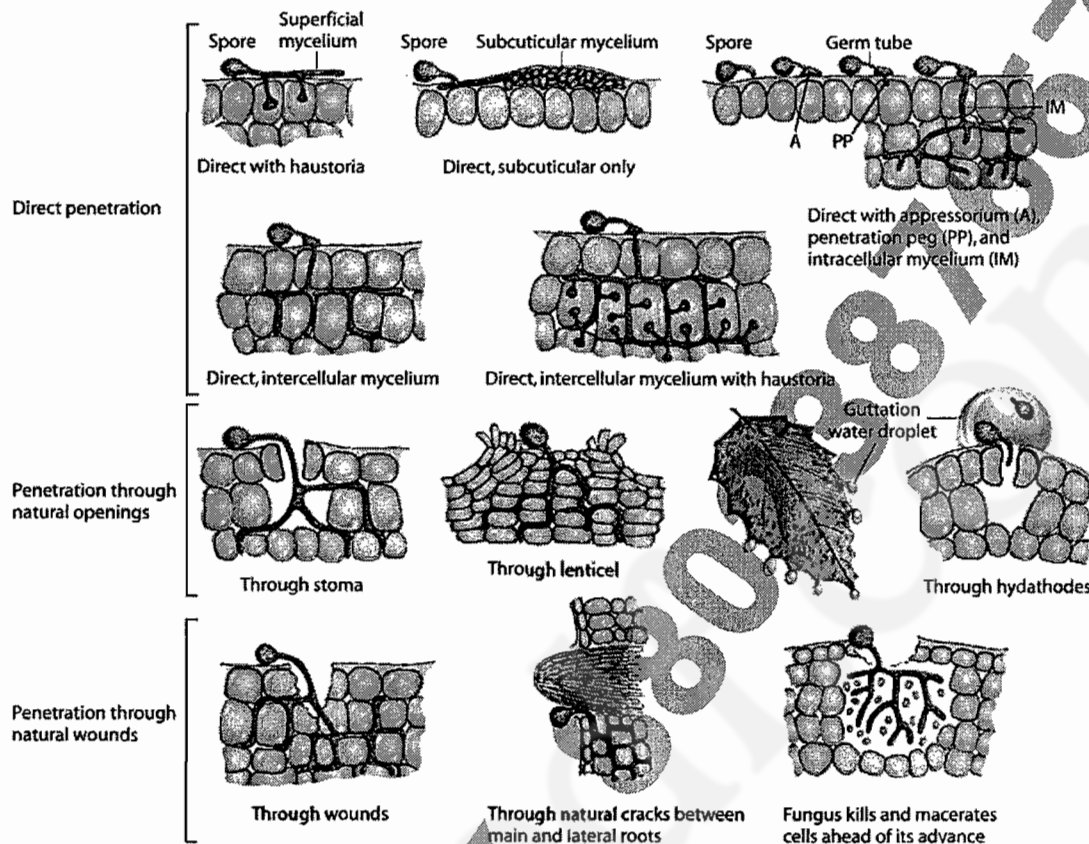


Figure: Methods of penetration by fungi

4. Bacteria enter plants mostly through wounds, less frequently through natural openings, and never directly through unbroken cell walls.

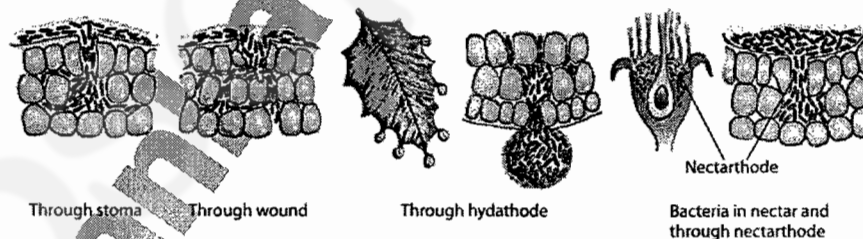


Figure: Methods of penetration and invasion by bacteria

5. Viruses, viroids, mollicutes, fastidious bacteria, and protozoa enter through wounds made by vectors, although some viruses and viroids may also enter through wounds made by tools and other means.
6. Parasitic higher plants enter their hosts by direct penetration.
7. Nematodes enter plants by direct penetration and, sometimes, through natural openings.

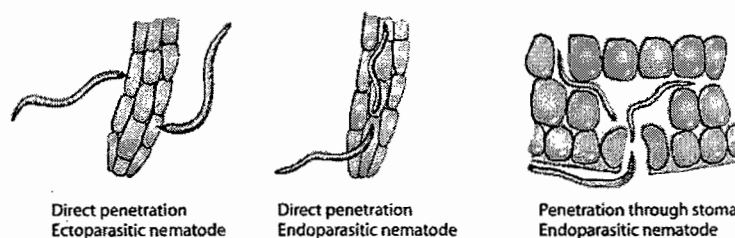


Figure: Methods of penetration and invasion by nematodes

8. **Recognition between Host and Pathogen:** It is still unclear how pathogens recognize their hosts and vice versa. It is assumed that when a pathogen comes in contact with a host cell, an early event takes place that triggers a fairly rapid response in each organism that either allows or impedes further growth of the pathogen and development of disease. The nature of the "early event" is not known with certainty in any host-parasite combination, but it may be one of many biochemical substances, structures, and pathways. These may include specific host signal compounds or structures, or specific pathogen elicitor molecules, and either of them may induce specific actions or formation of specific products by the other organism.
9. Penetration does not always lead to infection. Many organisms actually penetrate cells of plants that are not susceptible to these organisms and that do not become diseased; these organisms cannot proceed beyond the stage of penetration and die without producing disease.

Infection

Infection is the process by which pathogens establish contact with susceptible cells or tissues of the host and procure nutrients from them. Following infection, pathogens grow, multiply, or both within the plant tissues and invade and colonize the plant to a lesser or greater extent. Growth and/or reproduction of the pathogen (colonization) in or on infected tissues are actually two concurrent substages of disease development.

Successful infections result in the appearance of symptoms, i.e., discolored, malformed, or necrotic areas on the host plant. Some infections, however, remain latent, i.e., they do not produce symptoms immediately but at a later time when the environmental conditions or the stage of maturity of the plant become more favorable.

All the visible and otherwise detectable changes in the infected plants make up the symptoms of the disease. In most plant diseases, however, symptoms appear from a few days to a few weeks after inoculation. Symptoms may change continuously from the moment of their appearance until the entire plant dies or they may develop up to a point and then remain more or less unchanged for the rest of the growing season.

Symptoms may appear as soon as 2 to 4 days after inoculation, as happens in some localized viral diseases of herbaceous plants, or as late as 2 to 3 years after inoculation, as in the case of some viral, mollicute, and other diseases of trees.

The time interval between inoculation and the appearance of disease symptoms is called the **incubation period**. The length of the incubation period of various diseases varies with the particular pathogen-host.

During infection, some pathogens obtain nutrients from living cells, often without killing the cells or at least not for a long time; others kill cells and utilize their contents as they invade them; and still others kill cells and disorganize surrounding tissues. During infection, pathogens release a number of biologically active substances (e.g., enzymes, toxins, and growth regulators) that may affect the structural integrity of the host cells or their physiological processes. In response, the host reacts with a variety of defense mechanisms, which result in varying degrees of protection of the plant from the pathogen.

Invasion

Various pathogens invade hosts in different ways and to different extents.

1. Some fungi, such as those causing apple scab and black spot of rose, produce mycelium that grows only in the area between the cuticle and the epidermis (subcuticular colonization).
2. Others, such as those causing powdery mildews, produce mycelium only on the surface of the plant but send haustoria into the epidermal cells.
3. Most fungi spread into all the tissues of the plant organs (leaves, stems, and roots) they infect, either by growing directly through the cells as an intracellular mycelium or by growing between the cells as an intercellular mycelium.

4. Fungi that cause vascular wilts invade the xylem vessels of plants.
5. Bacteria invade tissues intercellularly, although when parts of the cell walls dissolve, bacteria also grow intra-cellularly.
6. Bacteria causing vascular wilts, like the vascular wilt fungi, invade the xylem vessels.
7. Most nematodes invade tissues intercellularly, but some can invade intracellularly as well. Several nematodes do not invade cells or tissues at all but feed by piercing epidermal cells with their stylets.
8. Viruses, viroids, mollicutes, fastidious bacteria, and protozoa invade tissues by moving from cell to cell intra-cellularly. Viruses and viroids invade all types of living plant cells, mollicutes and protozoa invade phloem sieve tubes and perhaps a few adjacent phloem parenchyma cells, and most fastidious bacteria invade xylem vessels and a few invade only phloem sieve tubes.
9. **Localised invasions:** Many infections caused by fungi, bacteria, nematodes, viruses, and parasitic higher plants are local, i.e., they involve a single cell, a few cells, or a small area of the plant. These infections may remain localized throughout the growing season or they may enlarge slightly or very slowly.
10. **Systemic invasions:** Infections caused by fastidious xylem- or phloem-inhabiting bacteria, mollicutes, and protozoa and natural infections caused by viruses and viroids are **systemic**, i.e., the pathogen, from one initial point in a plant, spreads and invades most or all susceptible cells and tissues throughout the plant. Vascular wilt fungi and bacteria invade xylem vessels internally, but they are usually confined to a few vessels in the roots, the stem, or the top of infected plants; only in the final stages of the disease do they invade most or all xylem vessels of the plant. Some downy mildew pathogens and some fungi, primarily among those causing smuts and rusts, also invade their hosts systemically, although in most cases the older mycelium degenerates and disappears and only the younger mycelium survives in actively growing plant tissues.

Growth and Reproduction of the Pathogen (Colonization)

Plant pathogens reproduce in a variety of ways.

1. Fungi reproduce by means of spores, which may be either asexual (mitospores, i.e., products of mitosis, roughly equivalent to the buds on a twig or the tubers of a potato plant), or sexual (meiospores, i.e. products of meiosis, roughly equivalent to the seeds of plants).
2. Parasitic higher plants reproduce just like all plants, i.e., by seeds.
3. Bacteria and mollicutes reproduce by fission in which one mature individual splits into two equal, smaller individuals.
4. Viruses and viroids are replicated by the cell.
5. Nematodes reproduce by means of eggs.

The rate of reproduction varies considerably among the various kinds of pathogens, but in all types, one or a few pathogens can produce tremendous numbers of individuals within one growing season.

Reproduction of the pathogen is followed by dissemination. An account of which is provided in the next section.

Dissemination of the Pathogen

Dissemination

All parasitic as well as viral diseases are transmissible. When a disease is established in a particular area, the transmission of the pathogen from one host to another or from one place to another is termed as dissemination. It is also called dispersal of the pathogen.

Dissemination can be typified in two ways.

1. Based on continuity
2. Based on mode

Dissemination types based on continuity

There are two main types of dissemination of plant pathogens on this basis.

1. **Continuous dissemination:** When dissemination occurs naturally by way of growth, multiplication and spread of the pathogen in an area where the disease is already established, it is called continuous dissemination.

2. **Discontinuous dissemination:** When the pathogen arrives in an area where a particular disease has never occurred, it is known as discontinuous dissemination. In such cases, the pathogen generally gets introduced through human activities, such as man carrying diseased material to a new locality or to a distant country for the purpose of introduction of new plants, crops, varieties etc.

Dissemination types based on mode

There are two main types of dissemination of plant pathogens on this basis.

1. **Direct transmission:** It is disease transmission where the pathogen is carried externally or internally on the seed or planting material like cuttings, sets, tubers, bulbs etc.
2. **Indirect transmission:** It means that the pathogen spreads itself by way of its persistent growth or certain structures of the pathogen carried independently by natural agencies like wind, water, animals, insects, mites, nematodes, birds etc.

Modes of Direct Transmission

1. **Internal transmission through seed or planting material:** False smut disease as well as Helminthosporium Blight disease of wheat are the common examples of fungal diseases carried internally through normal appearing seeds. Ring rot and Brown rot of potato caused by bacteria are carried internally through the tubers. Mosaic and leaf roll of potato which are viral diseases are also carried inside the infected tubers.
2. **External transmission through seed or planting material:** The common grain smut of jowar is an example of this type. The fungal structures called sclerotia in case of the Ergot disease of bajra are transmitted mostly in the form of physical mixture with the seed.

Modes of Indirect Transmission

1. **Autonomous transmission:** It takes place by continuous and persistent growth of the hyphae of the causal fungi in soil, characteristic of several wood rotting fungi attacking forest trees and some fruit plants.
2. **Wind dispersal:** Fungal spores produced externally on host surfaces are most easily carried by wind currents and this is the most dangerous mode of transmission of plant pathogenic fungi like those causing powdery and downy mildews, leaf spots, blasts, blights and rust diseases. The black stem rust disease of wheat in India perpetuates on wild grasses in the Nilgiri hills in the south India from where the rust spores are carried to south, central & then to north India by wind currents every year.
3. **Water dissemination:** This mode is comparatively less important than the wind transmission. Splashing rain drops mostly transmit the foliar diseases from leaf to leaf, from shoot to shoot and even from plant to plant in case of closely spaced crops. Plant pathogens requiring high humidity conditions like the fungi causing downy mildew diseases or bacteria causing canker of citrus are well adapted to this kind of short distance water dispersal.
4. **Farm Animals:** Farm animals are likely to carry the pathogen externally on their body surface, particularly on legs and hoofs, etc. or internally through their intestinal tract. Commonly, the soil inhabiting fungi causing rots and wilts are carried externally while certain smut fungi causing diseases to grain crops are transmitted through the intestinal tract.
5. **Birds:** Birds play a very minor role in disease transmission. But, they play an important role in the dispersal of seeds of parasitic plant *Loranthus* sp.
6. **Implements and Tools:** Farm implements used for cultivation of soil are often likely to transmit plant pathogens from one place to another. The pathogens in this case are usually carried in the form of bits of plant disease debris lying in the soil. Several viral diseases are disseminated through the budding and grafting operations.
7. **Insects:** Most of the viral diseases of plants are transmitted through the agency of different insects. Both types of insects viz. sucking and chewing or/biting are capable of transmitting viral diseases. Certain bacterial and several fungal pathogens are also known to be carried by insects.
8. **Mites:** Mites are wingless arthropods resembling ticks and having four pairs of legs and no antennae. It is suspected that some viral diseases of chillies, tomato, brinjal, etc. have vector relationship with mites.

9. **Nematodes:** Nematodes have been observed to transmit viral, bacterial and fungal plant diseases. Nematodes feeding externally on host plant roots cause injuries to roots which become the avenues for entrance of fungal and bacterial pathogens infecting plant roots. The Fan-leaf virus of grapevine is a well known example of transmission through a species of nematodes.
10. **Biological transmission:** Dodder, which is a parasitic plant, is known to transmit certain viral diseases.
11. **Human dispersal:** Man is often responsible for transmission of plant diseases in two ways viz.
 - a. Workers handling seedlings, other planting material or fruits are likely to get personally in contact with plant pathogens like fungi or bacteria. Then they act as a vector of the pathogen unknowingly.
 - b. Man carrying out the transport of infected seed, nursery stock or timber, etc. to new locations becomes a cause of pathogen introduction at a new location.

Modes of Dissemination of Plant Viruses

The plant viruses rely on damage in the cell wall to enter into a plant cell. This is achieved either by the vector or simply by mechanical damage to cells. The main modes of plant virus transmission are as follows.

11. **Seeds:** Seeds formed by virus infected plants may contain viruses. These viruses are present in the next generation right since the beginning, leading to early outbreaks of disease in new crops. About 100 viruses are known to be transmitted this way.
12. **Pollens:** Pollens formed by infected flowers contain viruses. The viruses transmitted by this method result into low levels of fruit set. In some cases it may spread from the fertilized flower into the remaining body of the mother plant. Example: *Prunus necrotic ringspot virus*.
13. **Vegetative propagation/grafting:** If the vegetative propagation material is not virus free, the resulting plants will also be virus infected.
14. **Bacteria:** In case of *Agrobacterium tumefaciens*, the Ti plasmid of this organism has been used experimentally to transmit virus genomes between plants.
15. **Fungi Vectors:** Fungi can insert their hypha into the plant cell and transmit the virus also. About 15 plant viruses are transmitted with help of fungal genera like *Oplidium*, *Polymyxa* and *Spongospora*.
16. **Nematode Vectors:** - About 20 plant viruses are transmitted by nematodes, especially by the nematode genera: *Longidorus*, *Paralongidorus*, and *Xiphinema*. The important viruses transmitted by nematodes are: Grape fan leaf virus and Tobacco ringspot virus.
17. **Insect vectors:** It is a particularly efficient means of virus transmission. Insects, which bite or suck plant tissues transmit viruses to new hosts. This is known as *non-propagative transmission*. Group III geminivirus are transmitted by insect vectors (leafhoppers or whiteflies) by this method. These viruses cause a great deal of crop damage in plants such as tomatoes, beans, squash, cassava and cotton.

However, in other cases (e.g. many plant *rhodoviruses*) the virus may also infect and multiply in the tissues of the insect (*propagative transmission*) as well as those of host plants. In these cases, the vector serves as a means not only of distributing the virus, but also of amplifying the infection.
18. **Mite Transmission:** Mites belonging to the family Eriophyidae have been shown to transmit at least 6 known viruses including the Wheat streak mosaic virus.
19. **Mechanical Transmission:** Mechanical transmission of viruses is the most widely used method for experimental infection of plants and is usually achieved by rubbing virus-containing preparations into the leaves. This is also an important natural method of transmission. Virus particles may contaminate soil for long periods and may be transmitted to the leaves of new host plants as wind-blown dust or as rain-splashed mud.

20. **Dodder Transmission:** The parasitic plant *Cuscuta* is a major source of infection for host angiosperms. This is known to transmit at least 16 different viruses.

Overwintering

Different groups of pathogens have different methods to overcome the unfavorable period. The annual plants die at the end of the growing season, as do the leaves and fruits of deciduous perennial plants. Thus, pathogens that attack annual plants and renewable parts of perennial plants have evolved mechanism by which they can survive the cold winters or dry summers that may intervene between crops or growing seasons.

1. **Fungi:** It has evolved a variety of mechanisms for persisting between the crops as :
 - a. **Perennial Plants:** Fungi overwinter as mycelium in diseased tissue e.g. cankers and as spores at or near the infected surface of the plant or on the bud scales.
 - b. **Deciduous Plants:** As mycelium or spores on fallen, infected leaves or fruits or on the bud scales.
 - c. **Annual Plants:** As mycelium infected plant debris, as resting or other spores and as sclerotia (hard masses of mycelium) in infected plant debris or in soil, seeds and propagative organs.
2. **Bacteria:** Similar to fungi i.e. in infected plants, seeds and tubers, in infected plant debris and for some in soil.
3. **Viruses, Viroids, Mollicutes, Fastidious Bacteria and Protozoa:** Survive only in living plant tissue such as tops and roots of perennial plants, vegetatively propagative organs, seeds of some host. A few viruses survive within their insect vectors, and some viruses and viroids may survive on contaminated tools and in infected plant debris.
4. **Nematodes:** Eggs in soil; Eggs or nematodes in plant roots or in plant debris. Some Nematodes produce juvenile stages or adults that can remain dormant in seeds or on bulbs for many months or years.
5. **Parasitic higher plants:** Survive either as seeds, usually in the soil or as their infective vegetative form on their host.

Chapter 4: Physiology of parasitism

An overview of parasitism

An organism that lives on or in some other organism on a *persistent basis* and obtains its food from the latter is called a **parasite**. The removal of food by a parasite from its host is called **parasitism**.

A **plant parasite** is an organism that becomes intimately associated with a plant on a *persistent basis* and **multiplies or grows at the expense of the plant**. The removal by the parasite of nutrients and water from the host plant usually reduces efficiency in the normal growth of the plant and becomes detrimental to the further development and reproduction of the plant.

In many cases, **parasitism is intimately associated with pathogenicity**, i.e., the ability of a pathogen to cause disease, as the ability of the parasite to invade and become established in the host generally results in the development of a diseased condition in the host.

Parasitism of cultivated crops is a common phenomenon. In North America, for example, more than 8,000 species of fungi cause nearly 100,000 diseases, and at least 200 bacteria, about 75 mollicutes, more than 1,000 different viruses and 40 viroids, and more than 500 species of nematodes attack crops. Although about 2,500 species of higher plants are parasitic on other plants, only a few of them are serious parasites of crop plants. A single crop, e.g., the tomato, may be attacked by more than 40 species of fungi, 7 bacteria, 16 viruses, several mollicutes, and several nematodes. This number of diseases is average as corn has 100, wheat 80, and apple and potato each are susceptible to about 80–100 diseases.

Fortunately, however, in any given location, only a fraction of the diseases affecting a crop are present and, in any given year, only a small number of these diseases become severe.

Range of plant parasites

Of the large number of groups of living organisms, only a few members of a few groups can parasitize plants: fungi, bacteria, mollicutes, parasitic higher plants, parasitic green algae, nematodes, protozoa, viruses, and viroids.

These parasites are successful because they can invade a host plant, feed and proliferate in it, and withstand the conditions in which the host lives.

Biotrophs, Semi-biotrophs and Necrotrophs

Some parasites, including viruses, viroids, mollicutes, some fastidious bacteria, nematodes, protozoa, and fungi causing downy mildews, powdery mildews, and rusts, are **biotrophs**, i.e., **they can grow and reproduce in nature only in living hosts**, and they are called **obligate parasites**.

Other parasites (most fungi and bacteria) can live on either living or dead hosts and on various nutrient media, and they are therefore called **nonobligate parasites**. Some nonobligate parasites live most of the time or most of their life cycles as parasites, but, under certain conditions, may grow saprophytically on dead organic matter; such parasites are **semi-biotrophs** and are called **facultative saprophytes**.

Others live most of the time and thrive well on dead organic matter (**necrotrophs**) but, under certain circumstances, may attack living plants and become parasitic; these parasites are called **facultative parasites**.

Mode of attack of parasites

Obligate and nonobligate parasites generally differ in the ways in which they attack their host plants and procure their nutrients from the host.

Many nonobligate parasites **secrete enzymes that bring about the disintegration of the cell components of plants** and these alone or with the toxins secreted by the pathogen result in the death and degradation of the cells. The invading pathogen then utilizes the contents of the cells for its growth. Many fungi and most bacteria act in this fashion, growing as necrotrophs on a nonliving substrate within a living plant. This mode of nutrition is like that of saprophytes.

All **obligate and some nonobligate parasites do not kill cells in advance but get their nutrients either by penetrating living cells or by establishing close contact with them**. The association of these pathogens with their host cells is an intimate one and results in continuous absorption or diversion of nutrients, which would normally be utilized by the host, into the body of the parasite. The depletion of nutrients, however, although it restricts the growth of the host and causes symptoms, does not always kill the host. In the case of obligate parasites, death of the host cells restricts the further development of the parasite and may result in its death.

Host range of parasites

Parasites differ with respect to the kinds of plants that they can attack, with respect to the organs and tissues that they can infect, and with respect to the age of the organ or tissue of the plant on which they can grow.

Some pathogens are restricted to a single species, others to one genus of plants, and still others have a wide range of hosts, belonging to many families of higher plants. Some pathogens grow especially on roots, others on stems, and some mainly on the leaves or on fleshy fruits or vegetables. Some pathogens, e.g., vascular parasites, attack specifically certain kinds of tissues, such as phloem or xylem. Others may produce different effects on different parts of the same plant. With regard to the age of plants, some pathogens attack seedlings or the young tender parts of plants, whereas others attack only mature tissues.

Many obligate parasites are quite specific as to the kind of host they attack, possibly because they have evolved in parallel with their host and require certain nutrients that are produced or become available to the pathogen only in these hosts. However, many viruses and nematodes, although obligate parasites, attack many different host plants.

Nonobligate parasites, especially root, stem, and fruit-attacking fungi, usually attack many different plants and plant parts of varying age, possibly because these pathogens depend on nonspecific toxins or enzymes that affect substances or processes found commonly among plants for their attack.

In any case, the number of plant species currently known to be susceptible to a single pathogen is smaller than the actual number in nature, as only a few species out of thousands have been studied for their susceptibility to each pathogen. Furthermore, because of genetic changes, a pathogen may be able to attack hosts previously immune to it. It should be noted, however, that each plant species is susceptible to attack by only a relatively small number of all known plant pathogens.

Damages caused by parasitism

Parasitism frequently plays an important, but not always the most important, role in pathogenicity. Usually no correlation exists between the degree of parasitism of a pathogen and the severity of disease it can cause, as many diseases caused by weakly parasitic pathogens are much more damaging to a plant than others caused even by obligate parasites. Moreover, certain pathogens, e.g., slime molds and those causing sooty molds, can cause disease by just covering the surface of the plant without parasitizing the plant.

In most plant diseases, the amount of damage caused to plants is often much greater than would be expected from the mere removal of nutrients by the parasite.

This additional damage results from substances secreted by the parasite or produced by the host in response to stimuli originating in the parasite.

Tissues affected by such substances may show increased respiration, disintegration or collapse of cells, wilting, abscission, abnormal cell division and enlargement, and degeneration of specific components such as chlorophyll.

Therefore, that the damage caused by a parasite is not always proportional to the nutrients removed by the parasite from its host. Pathogenicity, thus, is the ability of the parasite to interfere with one or more of the essential functions of the plant, thereby causing disease.

Effect of parasitism on plant physiology

When pathogens infect plants for obtaining food for themselves, they also interfere with different physiological functions of the plant. This leads to the development of different symptoms.

The effect of infection on host physiology can be described under the following heads.

Effect of Pathogens on Photosynthesis

1. Pathogens cause chlorosis, necrotic lesions or large necrotic areas on green plant parts which reduce photosynthetic area. Similarly, in leaf spot, blight and other kinds of diseases in which there is destruction of leaf tissue, e.g., in cereal rusts and fungal leaf spots, bacterial leaf spots, etc, photosynthesis is reduced because the photosynthetic surface of the plant is lessened.
2. Overall chlorophyll content of leaves is reduced.
3. There are direct toxins which inhibit photosynthetic electron transport.

Effect of Pathogens on Translocation of Water and Nutrients in the Host Plant

When a pathogen interferes with the upward movement of inorganic nutrients and water or with the downward movement of organic substances, diseased conditions arise in the part of plants denied these materials. The diseased parts are unable to carry out their own functions and will deny the rest of the plant their services or their products, thus causing disease of the entire plant.

It may affect the plant in the following ways:

1. Interference with upward Translocation of Water and Inorganic Nutrients
2. Effect on Absorption of Water by Roots
3. Effect on Translocation of Water through the Xylem
4. Increased Transpiration in case of leaf infection
5. Interference with Translocation of Organic Nutrients through the Phloem

Effect of Pathogens on Host Plant Respiration

1. Rate of respiration increases in infected cells. It continues to rise during multiplication and sporulation of the pathogen.
2. Accumulation and oxidation of phenolic compounds (many associated with defense mechanisms) during increased respiration. These substances are respiratory uncouplers.

Effect of Pathogens on Permeability of Cell Membranes

Changes in cell membrane permeability is among the first detectable responses of cells to infection. It leads to loss of electrolytes, i.e., of small water soluble ions and molecules from the cell.

Effects of Pathogens on Transcription and Translation

1. Pathogens affect transcription by changing the composition, structure or function of the chromatin associated with the cell DNA.
2. Infected plants contain higher levels of RNA causing increased transcription/protein synthesis in cells leading to synthesis of substances involved in defense mechanisms.

Effect of Pathogen on Plant Reproduction

Many pathogens have a direct adverse effect on plant reproduction because they attack and kill the flowers, fruits or seed directly, or interfere and inhibit their production.

Chapter 5: Molecular basis of infection and disease resistance and defence

Molecular basis of infection

Plants are constantly exposed to insects and microbes in nature. Hostile or benign in outcome, microbial and pest interactions with plants rely on a molecular dialogue between partners. Certain aspects of this interaction is well understood, for example the early interactions between the host and the pathogen. The process of early interactions between the host and the fungal pathogen is being described below.

Molecular basis of early interactions between the host and the fungal pathogen

The perception of signals from plant surfaces by pathogenic fungi seems to be the result of signaling pathways mediated by cyclic adenosine monophosphate (cAMP) and mitogen-activated protein kinase (MAPK).

In response to a signal from the host plant, e.g., the presence of a hydrophobic plant surface, which transmits a signal for appressorium formation, the fungus perceives the extracellular signal and its transmission via the plasma membrane and, as a first step, it accumulates intracellular signaling molecules and induces a phosphorylation cascade.

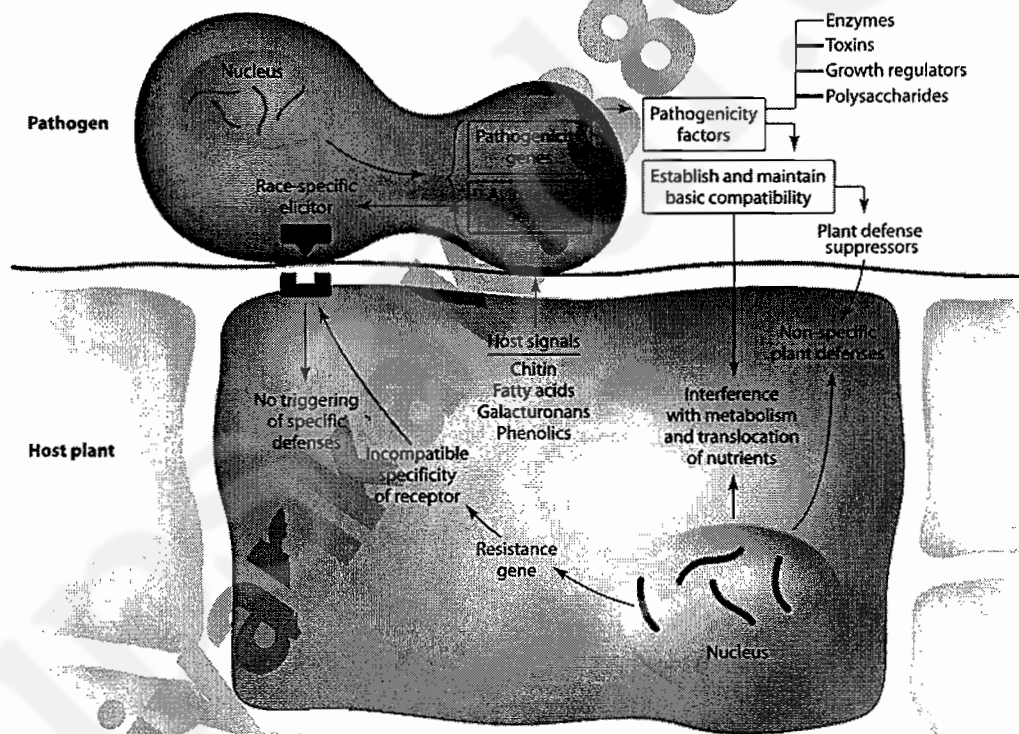


Figure: Establishment of infection in a compatible reaction between a pathogen and its host

In some fungi, the receptor of the signal is a protein in the plasma membrane of the fungal spore. Transmission of the cAMP signal proceeds via the cAMP-dependent activity of protein kinase A (= PKA) and subsequent phosphorylation of target proteins.

The major activity of PKA in developing germ tubes is the mobilization of carbohydrates and lipids to the appressorium site and is, therefore, pivotal to the production of functional appressoria. In some fungi, cAMP signaling is required for the initiation of appressorium development, at which time intracellular

cAMP concentrations rise during differentiation of conidia and emergence of the appressorium germ tube. Subsequently, cAMP levels fall as the germ tube extends and, if more cAMP is added at this point, further development of the germ tube is inhibited.

Signaling pathways for infection-related development are also achieved through mitogen-activated protein kinases (MAPKs) and their upstream regulatory kinases. All of these together comprise a functional unit that transmits input signals from the periphery of the cell to the cell nucleus to elicit the expression of appropriate genes. A MAP kinase, K1 or P1, regulates appressorium formation in response to a signal from the plant surface but it is also required for invasive growth or viability in its host plant.

Molecular basis of disease resistance and defence – I: Recognition of the Pathogen by the Host

Introduction

Plant species have numerous defence mechanisms of the induced type. For such a response, the first step necessary is the recognition of the pathogen by the host.

Pathogen recognition is the detection of some structural or molecular trait of the pathogen or the consequences of its penetration which lead to initiation of host's defence responses.

Mechanism

The pathogen recognition by the host is based on two components.

1. Pathogen elicitors
2. Host receptors

Pathogen elicitors

There are four types of elicitors.

1. Non specific elicitors: Mainly released by fungi and bacteria, such nonspecific elicitors include toxins, glycoproteins, carbohydrates, fatty acids, peptides, and extracellular microbial enzymes such as proteases and pectic enzymes.
2. Specific elicitors: In some host-pathogen combinations, certain substances secreted by the pathogen, such as *avr* gene products, *hrp* gene products, and suppressor molecules, act as specific pathogen elicitors.
3. Products of pathogen partial breakdown: In many cases, in which host enzymes break down a portion the pathogen surface, the wall polysaccharides act as elicitors for the host.
4. Products of host partial breakdown: When pathogen enzymes break down a portion of the plant surface polysaccharides, the released oligomers or monomers of the polysaccharides act as recognition elicitors for the plant.

Host Receptors

Host receptors are always proteins (mostly glycoproteins). They are mostly located outside or on the cell membrane, but some receptors occur intracellularly.

In the powdery mildew of cereals, a soluble carbohydrate that acts as an elicitor from the wheat powdery mildew fungus *Blumeria graminis* f. *sp. tritici* is recognized by a cytoplasmic receptor.

Operation of the recognition mechanism

Once the pathogen-derived elicitors are recognized by the host receptors, a series of alarm signals are sent to:

1. Host cell proteins
2. Nuclear genes

This signaling causes gene and protein activation and production of substances inhibitory to the pathogen. After this, the defence compounds mobilize toward the point of cell attack by the pathogen.

Some of the alarm substances and signal transductions are only intracellular, but in many cases the signal is also transmitted to several adjacent cells. The alarm signal may also be transmitted systemically.

The chemical nature of the transmitted signal molecules is not known with certainty in any host–pathogen combination. Several types of molecules have been implicated in intracellular signal transduction. The most common such signal transducers appear to be:

1. Various protein kinases
2. Calcium ions
3. Phosphorylases
4. Phospholipases
5. Atpases
6. Hydrogen peroxide (H_2O_2)
7. Ethylene

Systemic signal transduction, which leads to systemic acquired resistance, is thought to be carried out by:

1. Salicylic acid
2. Oligogalacturonides released from plant cell walls
3. Jasmonic acid
4. Systemin
5. Fatty acids
6. Ethylene

Molecular basis of disease resistance and defence – II: Hypersensitive Response (HR)

Introduction to Hypersensitive Response (HR)

Hypersensitive Response (HR) is a type of *necrotic biochemical and structural defence reaction* of plants to prevent the spread of infection by microbial pathogens. The HR is characterized by the rapid death of cells in the local region surrounding an infection. The HR serves to restrict the growth and spread of pathogens to other parts of the plant.

It is quite common in diseases caused by obligate fungal parasites, by viruses, bacteria and nematodes.

It is not yet clear whether the HR is the cause or the consequence of resistance.

Structural Features of HR

In many host–pathogen combinations, as soon as the pathogen establishes contact with the cell, the nucleus moves toward the invading pathogen and soon disintegrates. At the same time, brown, resin-like granules form in the cytoplasm, first around the point of penetration of the pathogen and then throughout the cytoplasm. As the browning discoloration of the plant cell cytoplasm continues and death sets in, the invading hypha begins to degenerate. In most cases the hypha does not grow out of such cells, and further invasion is stopped.

In bacterial infections of leaves, the hypersensitive response results in the destruction of all cellular membranes of cells in contact with bacteria, which is followed by desiccation and necrosis of the leaf tissues invaded by the bacteria.

Biochemical Aspects of HR

It is the result of recognition by the plant of specific signal molecules, the *elicitors*, produced by the avirulence gene of the pathogen and recognised by R-gene coded specific receptor molecules in the plant.

In nature, the elicitor molecule either reacts directly with the receptor protein, or releases compounds or reacts with another host protein (endogenous elicitors), which then interacts with the R-coded receptor.

Such recognition causes the activation of a cascade of host genes activated by signal transducers such as salicylic acid (SA), which result in a burst of oxidative reactions, disruption of cell membranes, and release of phenolic and other toxic compounds, which then lead to the hypersensitive response, programmed cell death, inhibition of pathogen growth, and thereby resistance.

The HR is often described in two phases.

1. In phase one of the HR, the activation of R genes triggers an ion flux, involving an efflux of hydroxide and potassium outside the cells, and an influx of calcium and hydrogen ions into the cell.
2. In phase two, the cells involved in the HR generate an oxidative burst by producing reactive oxygen species (ROS), superoxide anions, hydrogen peroxide, hydroxyl radicals and nitrous oxide. These compounds affect cellular membrane function, in part by inducing lipid peroxidation and by causing lipid damage.

The alteration of ion components in the cell, and the breakdown of cellular components in the presence of ROS, results in the death of affected cells and the formation of local lesions. Reactive oxygen species also trigger the deposition of lignin and callose, as well as the cross-linking of pre-formed hydroxyproline-rich glycoproteins to the cell wall matrix. These compounds serve to reinforce the walls of cells surrounding the infection, creating a barrier and inhibiting the spread of the infection.

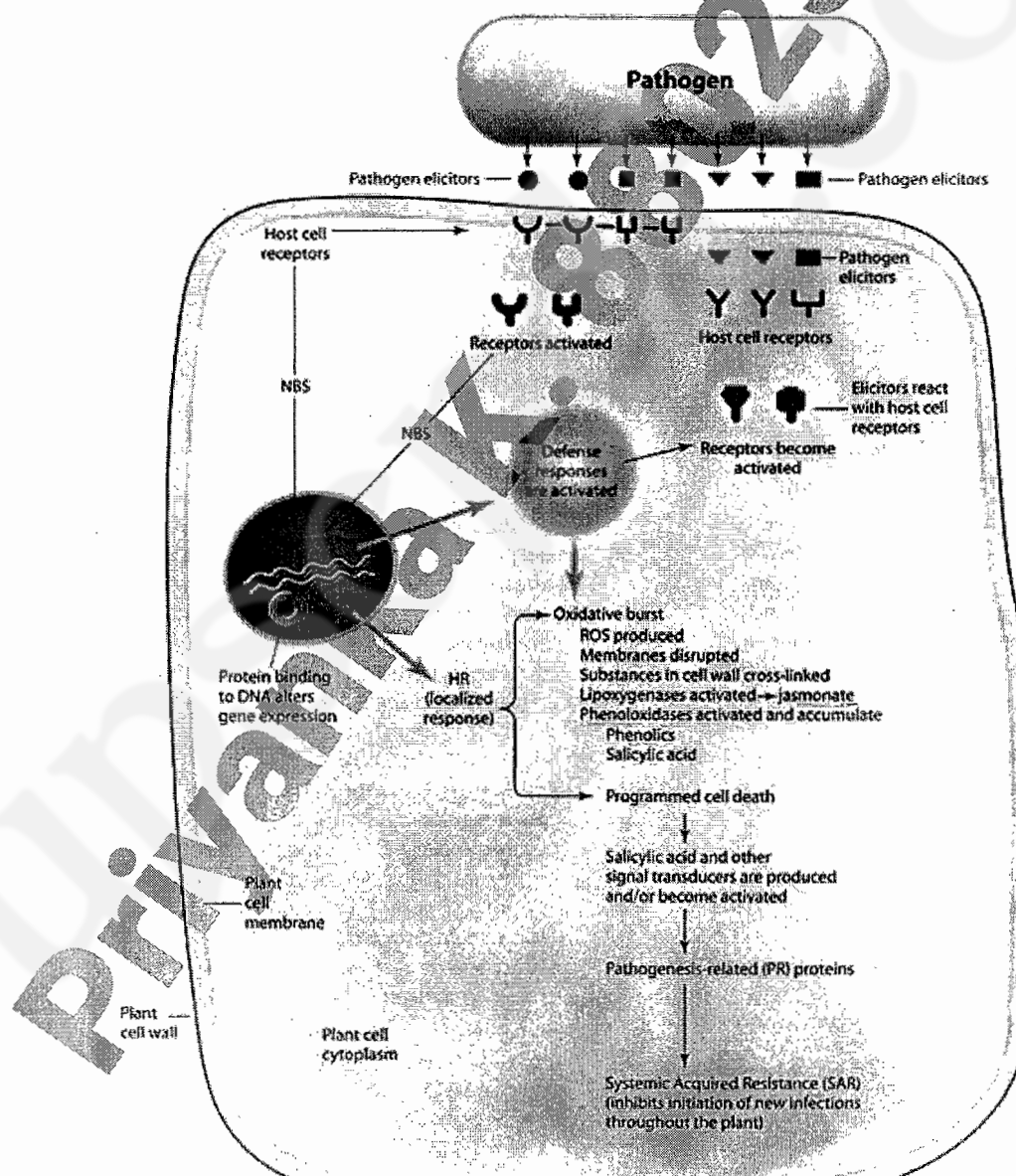


Figure: The schematic operation of HR

HR also leads to the activation of numerous other defense related genes that result in other types of resistance, including horizontal resistance and systemic acquired resistance (SAR). They comprise secondary resistance response induced after a hypersensitive response to avirulent pathogens.

Mediators of HR

Several enzymes have been shown to be involved in generation of ROS. They include:

1. Copper amine
2. Xanthine oxidase
3. NADPH oxidase
4. Oxalateoxidase
5. Peroxidases
6. Flavin containing amine oxidases

In some cases, the cells surrounding the pathogen synthesize antimicrobial compounds, including phenolics, phytoalexins, and pathogenesis related (PR) proteins, including α -glucanases and chitinases. These compounds may act by puncturing bacterial cell walls; or by delaying maturation, disrupting metabolism, or preventing reproduction of the pathogen in question.

Importance of HR

The HR creates necrotic tissue. It is significant for two reasons.

1. It isolates the parasite from the living substance on which it depends for its nutrition and, thereby, results in its starvation and death
2. It allows the concentration of numerous biochemical cell responses and antimicrobial substances that neutralize the pathogen. The faster the host cell dies after invasion, the more resistant to infection the plant seems to be.

Molecular basis of disease resistance and defence – III: Gene for Gene concept

Introduction to phytoimmunity

Plant resistance is also called *Phytoimmunity*. It is the state of having sufficient biological defences to avoid infection, disease, or other unwanted biological invasion. Phytoimmunity is thus a plant condition that is free from infection or simply, in a state of prolonged protection against diseases.

There are three main types of plant resistance against pathogens.

1. **Nonhost Resistance:** In such cases the plant under consideration is not host to a particular pathogen. In other words, the plant has some structural or biochemical trait due to which a particular pathogen cannot infect it at all. So the plant stays resistant even when it is brought in contact with a pathogen to which the plant is not a host. For example, apple trees are not affected by pathogens of tomato.
2. **Partial/Polygenic/Quantitative or Horizontal Resistance:** such kind of resistance depends on several genes. These genes code for various defence structures and chemicals which inhibit the pathogen. Such defence features may be pre-existing or induced.
3. **Race-Specific, Monogenic/R gene or Vertical Resistance:** The host plant carries one or few *Resistance* genes (*R*) per pathogen capable of attacking it, while each pathogen carries matching gene for *Avirulence* (*Avr*) for each of the *R* gene of host plant. This host-pathogen combination triggers a defence reaction (*Hypersensitive Response* or *HR*) that neutralizes the pathogen.

The Gene for Gene Concept

A general principle of plant resistance is that whatever the plant defence or resistance, it is controlled by its genes.

Infectious plant diseases are the result of the interaction of two organisms, the host plant and the pathogen. The properties of each of these two organisms are governed by their genetic make-up.

The **gene-for-gene concept** was given by Harold Henry Flor in 1955 (later refined in 1971) based on his studies on rust (*Melampsora lini*) of flax (*Linum usitatissimum*). This was the first model to study the genetics of both the host and parasite and to integrate them into one genetic system. This model is widely accepted as the valid explanation of the genetic basis of virulence in pathogens and of resistance in host plants.

It has been shown to operate in many other rusts, in the smuts, powdery mildews, apple scab, late blight of potato, and other diseases caused by fungi, as well as in several diseases caused by bacteria, viruses, parasitic higher plants, nematodes, and even insects.

Flor showed that the inheritance of both resistance in the host and virulence of the parasite is controlled by pairs of matching genes. One is a plant gene called the resistance (R) gene. The other is a parasite gene called the avirulence (Avr) gene. Plants producing a specific R gene product are resistant towards a pathogen that produces the corresponding Avr gene product.

The mechanism for host pathogen interaction based on R and Avr gene-pair is as follows.

Generally, in the host the genes for resistance are dominant (R), whereas genes for susceptibility, i.e., lack of resistance, are recessive (r). In the pathogen, however, genes for avirulence, i.e., inability to infect are usually dominant (Avr) whereas genes for virulence are recessive (avr).

Each gene in the host can be identified only by its counterpart gene in the pathogen, and vice versa.

Of the four possible gene combinations:

1. Only the Avr-R interaction is incompatible (resistant), i.e., the host has a certain gene for resistance (R) that recognizes the corresponding specific gene for avirulence (Avr) of the pathogen.
2. In the Avr-r combination, infection results because host lacks gene for resistance (r) and so the pathogen can attack it with its other gene for virulence.
3. In avr-R combination, infection results because although the host has a gene for resistance, the pathogen lacks the gene for avirulence that is recognized specifically by this particular gene for resistance and therefore no defense mechanisms (resistance) are activated.
4. In the avr-r interaction, infection results because the plant has no resistance (r) and the pathogen, being a pathogen and therefore virulent (avr), attacks it.

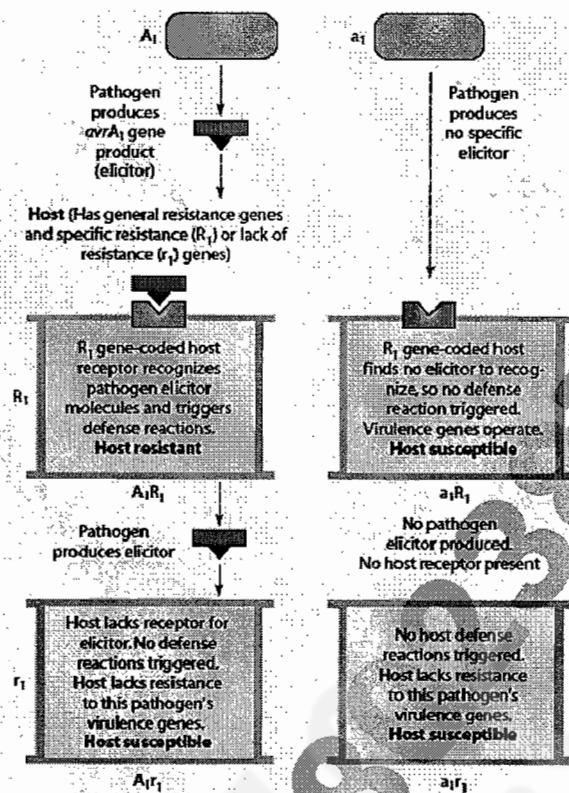


Figure 1: Mechanism for host pathogen interaction based on R and Avr gene-pair

In the recent years, several R and Avr gene products have been identified. The Avr gene products are either elicitors directly or they are enzymes responsible for elicitor synthesis.

In 1992, the first R gene, the maize *Hm1* gene, was located, isolated, and sequenced, and its function was described at the molecular level.

It is now known that all the R genes encode receptors in plants which carry out the recognition of pathogenic elicitors. Six classes of R gene encoded receptors are now well described.

Classes of Plant R Gene Proteins

Class	Function	Example of R gene
I	Membrane-associated, transcription regulating, mediating broad-spectrum resistance	RFW8
II	Cytoplasmic signal-transducing, serine-threonine protein kinase	Pto
III	Extracellular LRRs with transmembrane anchor	Cf-2-Cf-9
IV	Extracellular LRRs, with a transmembrane receptor and a cytoplasmic serine-threonine kinase	Xa21
V	Cytoplasmic, membrane associated. Contain LRRs, NBS, and TIR domains	RPP5, N, L6 _{maize}
VI	Also cytoplasmic, membrane associated. Contain LRRs, NBS, and a coiled coil domain	RPM1, RPS2

How do R genes confer resistance?

Elicitor molecule produced by Avr gene of pathogen recognised by receptor encoded R gene



One or more kinase enzymes activated



Phosphorylation and activation/energising of other kinases and other enzymes



A cascade of biochemical reactions



Hypersensitive Response, i.e., localised host resistance at the point of attack by the pathogen



Systemic Acquired Resistance (SAR) (is a secondary resistance response induced after a hypersensitive response to avirulent pathogens)

Pathogenicity Genes in Plant Pathogens

Pathogenicity factors encoded by "pathogenicity genes" (*pat*) and "disease specific genes" (*dsp*) are involved in steps crucial for the establishment of disease.

Pathogenicity genes are genes that make a particular microorganism a pathogen, i.e., capable of causing disease. Virulence/Avirulence genes act on top of the general pathogenicity of pathogen.

Example – Pathogenicity Genes of Fungi control production of infection structures, degradation of cuticle and cell wall, control secondary metabolite compounds called *phytoanticipins* and *phytoalexin*, control production of fungal toxins and genes involved in signalling mechanism.

Signal Transduction between Pathogenicity Genes and Resistance Genes

Induced defenses of plants against pathogens are regulated by networks of interconnecting signalling pathways in which the primary components are the plant signal molecules Salicylic Acid (SA), jasmonic acid (JA), ethylene (ET), and nitric oxide (NO). In plant-pathogen interactions, plants react to attack by pathogens with enhanced production of these substances while a distinct set of gene-to-gene resistance defense-related genes is activated and attempts to block the infection.

Chapter 6: Fungal Toxins

Introduction to Fungal Toxins

Fungal Toxin or **Mycotoxins** are toxic fungal metabolites which cause disturbance in host's physiological processes. They are injurious to plants and directly and indirectly play a role in disease development. The Toxins act directly on living host protoplasts, seriously damaging or killing the cells of the plant.

Toxins injure host cells by:

1. affecting the permeability of the cell membrane
2. inactivating certain enzymes and subsequently interrupting the corresponding enzymatic reactions
3. acting as antimetabolites and induce a deficiency for an essential growth factor.

Types of fungal toxins

There are two basic types of mycotoxins.

1. Toxins affecting a wide range of host plants
2. Host-specific or host-selective toxins

1. Toxins that affect a wide range of host plants

These toxic substances affect not only on the host plant but also some non-host species of plants. Some examples are described as follows.

Tentoxin

Tentoxin is a very well studied toxin in this category. It is produced by the fungus *Alternaria alternata* (previously called *Alternaria tenuis*), which causes chlorosis in seedling plants of many species. Tentoxin is a cyclic tetrapeptide that binds to and inactivates a protein (chloroplast-coupling factor or Cp-ATP Synthase complex) involved in ATP synthesis. In sensitive species, tentoxin interferes with normal chloroplast development and chlorophyll synthesis, thus leading to chlorosis.

Other Non-Host-Specific Toxins

1. Fumaric acid, produced by *Rhizopus spp.* in the almond hull rot disease;
2. Oxalic acid, produced by *Sclerotium* and *Sclerotinia spp.* in various plants they infect,
3. Zinniol and Alteraric acid produced by *Alternaria spp.* in leaf spot diseases of various plants
4. Ceratoulmin, produced by *Ceratocystis ulmi* in Dutch elm disease
5. Fusicoccin, produced by *Fusicoccum amygdali* in the twig blight disease of almond and peach trees
6. Ophiobolin, produced by several *Cochliobolus spp.* in blight diseases of grain crops;
7. Pyricularin, produced by *Pyricularia oryzae* in the rice blast disease
8. Cercosporin, produced by *Cercospora spp.* in leaf spots of several plants
9. Fusaric acid and Lycomarasmin, produced by *Fusarium oxysporum* tomato wilt; and many others.

2. Host-specific or host-selective toxins

Host-specific toxins have been show to be produce only by certain fungi belonging to the genera *Cochliobolus*, *Alternaria*, *Periconia*, *Phyllosticta*, *Corynespora*, and *Hypoxylon*. A host-specific toxin is toxic only to the hosts of that pathogen and shows little or no toxicity against nonsusceptible plants. Some important examples are listed below.

Victorin, or HV Toxin

Victorin, or HV-toxin, is produced by the fungus *Cochliobolus (Helminthosporium) victoriae*. This fungus infects the basal portions of susceptible oat plant and produces a toxin that is carried to the leaves, causes leaf blight, and destroys the entire plant.

Victorin is a partially cyclic pentapeptide. The primary target of the toxin is the cell plasma membrane where Victorin binds to several proteins and disturbs their function.

T-Toxin [*Cochliobolus (Helminthosporium) heterostrophus* Race T Toxin]

T-Toxin is produced by race T of *Chchliobolus heterostrophus*, the cause of southern corn leaf blight. T-Toxin is a mixture of linear, long (35 to 45 carbon) polyketols. T-Toxin apparently acts specifically on mitochondria of susceptible cells, which it renders nonfunctional, and inhibits ATP synthesis. The T-toxin reacts with a specific receptor protein molecule that is located on the inner mitochondrial membrane of sensitive mitochondria.

HC-Toxin

Cochliobolus (Helminthosporium) carbonum causes a leaf spot disease in maize and produces the host-specific HC-toxin, which is toxic only on specific maize lines. The mechanism of action of HC-toxin is not known, but this is the only toxin, so far, for which the biochemical and molecular genetic basis of resistance against the toxin is understood. Resistant corn lines have a gene (HM1) coding for an enzyme called HC-toxin reductase that reduces and thereby detoxifies the toxin. Susceptible corn lines lack this gene and, therefore, cannot defend themselves against the toxin.

AM-Toxin

AM-Toxin is produced by the apple pathotype of *Alternaria alternate*, the cause of Alternaria leaf blotch of apple. The toxin molecule is a cyclic peptide, and it usually exists as a mixture of three forms. The toxin is extremely selective for susceptible apple varieties. The AM- toxin causes plasma membranes of susceptible cell to develop invaginations, and cells show significant loss of electrolytes. AM-toxin also causes rapid loss of chlorophyll, suggesting that this toxin has more than one site of actions.

Other Host-Specific Toxins

Numerous additional host-specific toxins are known, and many more will undoubtedly be discovered in the future. Several of the additional toxins are also produced by species of the fungi *Cochliobolus* or *Alternaria*; HS-toxin; produced by *Alternaria citri* (lemon race,) affects rough lemon; ACT-toxin, produced by *A. citri* (tangerine race) affects Dancy tangerine; AL-toxin, produced by *A. alternate lycopersici* affects tomato; other *Alternaria* species also produced AF-toxin on strawberry, AK-toxin of Japanese pear, and AT-toxin on tobacco.

At least two other fungi produce well-known host-specific toxins; *Periconia circinata* produces the PC-toxin in the sorghum, and *Phyllosticta maydis* produces the PM-toxin in corn that has Texas male-sterile cytoplasm. Another fungus, *Corynespora cassicola*, produces the CC-toxin in tomato. Toxin produced by some other fungi, for example, *Hypoxyton mammatum* on poplar and *Perenophora teres* on barley seem to be species –selective rather than host-specific.

Chapter 7: Control measures for plant diseases

An overview

Control increases the quantity and improves the quality of plant products available for use. Methods of control vary considerably from one disease to another, depending on the kind of pathogen, the host, the interaction of the two, and many other variables. In controlling diseases, plants are generally treated as populations rather than as individuals.

Types of control measures

Various control measures can be classified as:

1. **Regulatory Control Measures** – aim at excluding a pathogen from a host or from a certain geographic area. Such regulatory control is applied by means of – (i) quarantines, (ii) inspections of plants in the field or warehouse, (iii) voluntary or compulsory eradication of certain host plants. The above 3 measures can be further shown to include –
 - Crop Certification – indicating that plants are free from certain diseases.
 - Evasion or avoidance of pathogen – through crop isolation
 - Use of pathogen free propagating material – such as seed, vegetative propagating material, and exclusion of pathogens from plant surfaces by epidermal coatings.

Example: To produce potato seed tubers free of viruses, potatoes are grown in remote locations in the cooler, northern states and at higher elevations, where aphids, the vectors of these viruses, are absent or their populations are small and can be controlled.

2. **Cultural Control Measures** aim at helping plants avoid contact with a pathogen, creating environmental conditions unfavorable to the pathogen or avoiding favourable ones, and eradicating or reducing the amount of a pathogen in a plant, a field or an area. They depend primarily on certain actions of the grower, such as –
 - Host Eradication – eg. – Host eradication controlled the bacterial citrus canker in Florida in 1915, where 3 million trees had to be destroyed.
 - Crop Rotation – Planting crops, for 3 or 4 years, belonging to species or families not attacked by the particular soil-borne pathogen. Eg. – Some diseases such as stalk rot of grain sorghum and corn by *Fusarium moliforme*, reduced through crop rotation.
 - Sanitation – consists of all activities aimed at eliminating or reducing the amount of inoculum present in a plant, a field, or a warehouse and at preventing the spread of the pathogen to other healthy plants and plant products. Eg. – removing infected leaves of house or garden plants.
 - Improving Plant Growing Conditions
 - Creating Conditions Unfavourable to Pathogens – Eg. – Spacing plants properly in field prevents creation of high humidity conditions and inhibits infections by pathogens as *Botrytis*.
 - Polyethylene Traps & Mulches – Eg. – Vertical, sticky, yellow polyethylene sheets erected along the susceptible pepper crops attract airborne aphid vectors carrying viruses such as cucumber mosaic virus which stick to the plastic and thus inoculum is prevented from reaching the crop.
 - Trickle Irrigation
 - Ecofallow
 - Reduced Tillage Farming (sometimes)
3. **Biological Control Measures** aim at improving the resistance of the host or favoring microorganisms antagonistic to the pathogen. These methods use living organisms to reduce the pathogen inoculum, such as –
 - Use of Trap Crops and Antagonistic Plants against Nematodes

- Use of amendments that favour Microflora Antagonistic to the Pathogen
- Use of Antagonistic Microorganisms - Antagonists, through the antibiotics they produce, through lytic enzymes, through competition for food, or through direct parasitizing of the pathogen, do not allow the pathogens to reach high enough populations to cause severe disease.

Eg. - (a) Biological control of weeds through pathogens that infect, damage, and may even kill weeds is a promising area of plant pathology. (b) Soil amended with soil containing a strain of a *Streptomyces* species antagonistic to *Streptomyces scabies*, the cause of potato scab, resulted in potato tubers significantly free from potato scab. (c) Bacteria of the genera *Bacillus*, *Enterobacter*, *Pseudomonas*, and *Pantoea* parasitize &/or inhibit the pathogenic oomycetes *Phytophthora* sp., *Pythium* sp. Etc.

4. **Transgenic Technology as a Control Method** - a type of biological control involving the transfer of genetic material (DNA) into plants that generate transgenic plants that exhibit resistance to a certain disease(s) by producing enzymes, peptides, or toxins interfering with infection by the pathogen.

Techniques involved -

- Transgenic Plants that tolerate Abiotic Stresses
- Transgenic Plants Transformed with Specific Plants genes for Resistance
- Transgenic Plants Transformed with Genes Coding for Anti-pathogen compounds
- Transgenic Plants Transformed with Nucleic Acids that Lead to Resistance and to Silencing of Pathogen Genes
- Transgenic Plants Transformed with Combinations of Resistance Genes
- Transgenic Plants Producing Antibodies Against the Pathogens

Eg. - (a) Several plant viruses have been found suppressed in transgenic plants transformed with genes that enable them to produce antibodies such as *TMV*, *potato virus X*, *clover yellow vein virus*. (b) Eggplant transformed with the bacterial gene coding for mannitol phosphodehydrogenase are tolerant against osmotic stress induced by salt, against drought, and against low temperatures. (c) Peanuts plants transformed with antifungal genes that reduced the incidence of *Sclerotinia* blight, caused by *Sclerotinia minor*, by 36% compared to susceptible nontransgenic plants.

5. **Physical Methods of Control** depend on physical factors such as heat or cold, dry air, unfavourable light wavelengths. Techniques include -

- Soil Sterilization Eg. - Soil can be sterilized by heat in greenhouses.
- Heat Treatment (Air or Water) of Plant Organs - Eg. - Treatment of bulbs and nursery stock with hot water frees them from nematodes that may be present within them, such as *Ditylenchus dipsaci* in bulbs of various ornamentals.
- Eliminating Certain Light Wavelength - Eg. - *Alternaria*, *Botrytis* - plant pathogenic fungi that sporulate only when they receive light in the UV range. Greenhouses have been constructed that absorb UV light and thus control disease.
- Refrigeration - most widely used and the most effective method of controlling post harvest diseases of fleshy plant products by greatly inhibiting or retarding the growth and activities of pathogens.
- Radiation - Eg. - Electromagnetic radiations such as UV light, X-rays, and γ -rays control post harvest infections of peaches, tomatoes by fungal pathogens.

6. **Chemical Methods of Control** aim at protecting the plants from pathogen inoculum that has arrived, or is likely to arrive, or curing an infection that is already in progress.

These depend on the use and action of a chemical substance to reduce the pathogen, such as -

- Soil Treatment with Chemicals - soil is treated with chemicals for control primarily of nematodes but occasionally also of soil-borne fungi, such as *Fusarium* and *Verticillium*, weeds and bacteria. Fungicides used for soil treatment include metalaxyl, diazoben, pentachloronitrobenzene (PCNB), etc.
- Soil Fumigation - Chloropicrin, methyl bromide, etc. are fumigants that either volatilize or decompose into gases in the soil - effective against wide range of microbes.
- Disinfestation of Warehouses - Eg. - Fumigation of warehouses

- Control of Insect Vectors – Eg. – Control of aphid-borne viruses by oils prays has been successful with some viruses (eg. Cucumber mosaic virus on cucumber).

Concluding remarks

In general, excluding or reducing the initial inoculum is most effective for the management of monocyclic pathogens. With polycyclic pathogens, a reduction in the initial inoculum must be accompanied by a control measure that reduces the infection rate.

The genetic engineering approach will improve resistance in susceptible plants, in combination with conventional plant breeding, and thus provide one of the most effective tools for controlling plant diseases.

Chapter 8: Modelling and disease forecasting

Modelling of Plant Disease Epidemics

What is an epidemic?

When a pathogen spreads to and affects many individuals within a population over a relatively large area and within a relatively short time, the phenomenon is called an epidemic.

What does plant disease modelling mean?

The mathematic relationship that describes the interaction between the environment, host and pathogen variable, and the disease is described as the model and is presented as an equation, as a graph, as a table or as a simple statement.

Since late 1960s plant pathologists have been developing models of the most common and serious diseases.

Components of an Epidemic

5 interacting components of an Epidemic are host, pathogen, environment, time and human.

Host Factors affecting the development of Epidemics

- Levels of genetic Resistance or Susceptibility of the Host
- Degree of Genetic Uniformity of Host Plants
- Type of Crop
- Age of Host Plants

Pathogen factors affecting the development of Epidemics

- Levels of Virulence
- Quantity of Inoculum Near Hosts
- Type of Reproduction of the Pathogen
- Ecology of the Pathogen
- Mode of Spread of the Pathogen

Environmental factors affecting the development of Epidemics include factors such as moisture, temperature, etc.

Effect of human cultural practices and control measures

- Site Selection and Preparation
- Selection of Propagative Material
- Cultural Practices
- Disease Control Measures
- Introduction of New Pathogens

How is a model developed?

Humans have been extremely interested in determining the elements and conditions that initiate the appearance, development, and spread of epidemics, the conditions that influence the rate of increase and the direction of their path, and the conditions that bring about their demise. For these, observations, measurements, mathematical formulas, and computers are used extensively to study the development and to predict the size, path, and time of attack in any given location.

Each plant disease epidemic, eg., stem rust of wheat, late blight of potato, etc. follows a predictable course in each location each year. The course of the epidemic varies with the host varieties and pathogen races present and other factors influencing host-pathogen interaction (mentioned above).

The construction of a model takes into account all of the components and as many subcomponents of a specific plant disease for which there is information for quantitative treatment, i.e., for treatment with mathematical formulas. The models constructed are generally crude simplifications of real epidemics. The

more accurately the real subcomponents of an epidemic are measured and fitted together, the more accurately they describe the epidemic.

In developing a plant disease model, a database of information is developed about as many of the components of a plant disease as possible. The database contains information on the crop, the disease, the pathogen, the location of the weather station, and sensor(s) related to the crop and the crop canopy. The database also contains information on the input variables such as measured environmental variables, host and pathogen variables. Thus a mathematical relationship is developed.

Use of Modern Technology in Plant Disease Modelling

The availability of computers has allowed plant pathologists to write programs that allow the simulation of epidemics of the most important diseases.

Examples of a few disease modelling programs – (a) EPIDEM – (1) resulted from modelling each stage of the life cycle of a pathogen as a function of the environment. (2) designed to simulate epidemics of early blight of tomato and potato caused by *Alternaria solani*. (b) EPICORN – for southern corn leaf blight caused by *Cochliobolus*.

Other New tools in Modelling – Molecular tools, GIS, Remote Sensing, GPS, Geostatistics, Image Analysis and IT.

Merits of Disease Modelling

- Allows determining whether, and when, to intervene with control measures.
- Allows determining what types of disease management strategies can be employed to slow down, or entirely prevent, the disease in a particular location.

Caution

Because plant disease models are developed for specific climates and regions, a model not developed in a specific area must be tested and validated for a specific location for one or more reasons to verify that it will work in this location.

Disease forecasting

Disease forecasting means using the success of disease modelling or computer simulation to predict the intensity, path of the disease or simply put, predict the whether, when, where, and how of a plant disease.

To develop a plant disease forecast, one must take into account several characteristics of the particular pathogen, host and environment. In general,

- a. For most monocyclic diseases (such as root rot of peas and Stewart's wilt of corn) and for a few polycyclic diseases that may have a large amount of initial inoculum (such as apple scab), disease development may be predicted by assessing the amount of initial inoculum.
- b. For polycyclic diseases (such as late blight of potato) that have a small amount of initial inoculum but many infection cycles, disease development can best be predicted by assessing the rate of occurrence of the infection cycles.
- c. For diseases in which both the amount of initial inoculum and the number of disease cycles are large (such as beet yellows) both factors must be assessed for the accurate prediction of disease epidemics.

Merits of Disease Forecasting

- a. Extremely useful to farmers in the practical management of crop disease.
- b. Disease forecasting allows the prediction of probable outbreaks or increases in intensity of disease and, therefore, allows to determine whether, when, and where a particular management practice should be applied

Role of New Technology as Tools in Forecasting

- a. Computer Simulation is the most important tool in disease forecasting. Expert systems are computer programs that try to equal or surpass the logic and ability of an expert professional and in solving problems. They are frequently used for diagnostic purposes, i.e., identifying the cause of

a disease by the symptoms and related observations. Eg. BLITECAST – a computerized forecasting system for potato late blight.

- b. Molecular Tools – Development and use of genetic (DNA) probes to detect and identify a plant pathogen and changes in pathogen.
- c. Global Positioning System (GPS) – enables one to pinpoint an individual tree or a specific area or areas affected by a pathogen. Consequently, the affected area or part can be treated rather than the whole field. Also used to apply pesticides, nutrients called Precision Farming.

A few Examples

- a. Forecasts based on Amount of Initial Inoculum – In Stewart's wilt of corn (caused by bacterium *Erwinia stewartii*), the pathogen survives the winter in the bodies of its vector, the corn flea beetle. These beetles are killed in prolonged low winter temperatures. Therefore, the amount of disease that will develop in a growing season can be predicted if the number of vectors that survived the winter is known, as that allows an estimation of the amount of inoculum that also survived the winter.
- b. Forecasts based on Weather conditions favoring development of Secondary Inoculum – In late blight of tomato and potato (caused by *Phytophthora infestans*), the initial inoculum is usually low and too small to detect directly. Weather conditions – constant cool temperature – favour development of secondary inoculum and lead to epidemic. Computerized predictive systems – BLITECAST – for late blight ; FAST – for forecasting *A.Solani*– developed.

Chapter 9: Plant Quarantine

An overview of plant quarantine

Plant quarantine is a mean by which plant materials are kept in isolation to prevent the spread of disease etc. present in them to the other plant materials. It is vital to prevent the introduction of non-indigenous, potentially damaging pests and diseases of plants into a country or to eradicate them before they can become widespread and well established. Less-developed countries and other countries in transition are especially vulnerable to the damaging effects of exotic pest introductions because of often inadequate infrastructure and the fragility of their economies and the importation of germplasm for agricultural development. Some of the worst plant disease epidemics, e.g. the downy mildew of grapes in Europe and the citrus canker, chestnut blight, Dutch elm disease and soybean cyst nematode in the United States, are all disease caused by pathogens that were introduced from abroad.

In India also, several pests and diseases got introduced from time to time, some of which, like late blight of potato, banana bunchy top, bacterial blight and streak diseases of paddy, have since become widespread. Some others like golden nematode and wart disease of potato and downy mildew of onion are still localized in certain parts of the country. Thus, plant quarantine, in real sense, serves as a national service by preventing the introduction of exotic pests/pathogens/weeds and their further spread. However, such endeavours could succeed only with the active support of all-the administrators, general public, farmers, scientists, communication media, customs and others.

To check the disease spread, in the cases of plant introduction, all the introduced plant propagules are thoroughly inspected for contamination with weeds, disease and insect pests. Plant material is fumigated or is given other treatments to get rid of the contamination. Sometimes the materials are grown in isolation for observation of diseases, insects, pests and weeds. This entire process is known as quarantine and the rules as quarantine laws. According to the quarantine laws, only those propagules that are free from diseases, insect pests and weeds can be allowed to enter the country. The quarantine laws cover not only the propagules but also their packing materials and other materials accompanying them.

In India 3 agencies have been involved to quarantine the plant propagule, depending on the nature of the concerned species:

1. NBPGR : Checks all propagules of agricultural and horticultural species.

2. FRI, Dehradun : Quarantine propagules of forest trees.
3. BSI, Calcutta : Quarantine remaining plant species.

Quarantine Procedure

NBPGR takes the following necessary steps to prevent the entry of weeds, diseases and pests. It takes around three weeks to quarantine the plant. The quarantine of short -lived propagules is done at top priority.

1. It is essential that the propagules must be clean, healthy and free from weeds and insect pests. They must not be treated by the sender with fungicides or insecticides. If necessary, the sender may only fumigate the seeds/propagules before sending them, and indicate the same in the phytosanitary certificate.
2. Each imported entry or sample must be accompanied by a 'phytosanitary certificate' from the scientist/institution sending the sample/entry. In this certificate the sender certifies that the seeds/propagules being sent are free from weeds, diseases and pests. All the entries not accompanied by an authentic phytosanitary certificate are either returned to the sender or destroyed by the bureau.
3. The entries accompanied by an authentic phytosanitary certificate are examined closely with the help of a magnifying glass/microscope and screened with X-rays. X-ray examination is helpful in the detection of insects etc. within the propagules.
4. The healthy entries free from diseases, insect pests and weeds are identified and sent to the recipient scientists/institutions. Contaminated entries are detained by the bureau and attempts are made to free them from the contaminating weeds, insects and pathogens.
5. Contaminated entries may be fumigated if it is considered that such a treatment would rid them of the contaminating insect or pathogen
6. If needed or considered desirable, the contaminated entries may be grown in isolation in an effort to isolate some healthy plants. Seeds/propagules from such healthy plants may be collected and sent to the indenter.
7. Samples/entries that are heavily contaminated are destroyed by the bureau.

Chapter 10: Miscellaneous topics in plant pathology

1. Phytoimmunity

Immunity is defined as the state of having sufficient biological defenses to avoid infection, disease, or other unwanted biological invasion.

Phytoimmunity is a plant condition that is free from infection or simply, in a state of prolonged protection against diseases.

Types

Immunity in plants is categorised into two –

1. Innate
2. Acquired

Each of the above two categories is further divided into –

1. **Structural characteristics** act as physical barriers and inhibit the pathogen from gaining entrance and spreading through the plant.
2. **Biochemical reactions** take place in the cells and tissues of the plant and produce substances that either toxic to the pathogen or create conditions that inhibit growth of the pathogen in the plant such as –
 - a rapid burst of rapid oxygen species, leading to dramatic increase of oxidative reactions;
 - increased ion movement from the cell membrane;
 - disruption of membranes and loss of cellular compartmentalization;
 - transient action of protein kinases;
 - production of antimicrobial substances such as phenolics (phytoalexins); &
 - formation of antimicrobial pathogenesis-related proteins such as chitinases.

Innate immunity

Structural Characteristics

- First line of defence of a plant against pathogens is its surface.
- Structural defenses in plant before the pathogen comes in contact with the plant – include the amount and quality of wax and cuticle that cover the epidermal cells, the structure of the epidermal cell walls, the size, location and shapes of stomata and lenticels, and the presence of tissues made of thick-walled cells.

Biochemical Defences

- Plants exude a variety of substances through surface of – above ground parts and through the surface of their roots.
- Some plants are resistant to diseases caused by certain pathogens because of one or more inhibitory antimicrobial compounds, called *phytoanticipins* – present in the cell before infection.
- Several **phenolic compounds**, tannins, and some fatty acid-like compounds such as dienes, which are present in high concentrations in cells of young fruits, leaves, or seeds, have been proposed as responsible for the resistance of young tissues to pathogenic microorganisms such as *Botrytis*.

Acquired immunity

Various pathogens, especially fungi and bacteria, release a variety of substances – non-specific elicitors – in their immediate environment for the host to recognise. These elicitors include toxins, glycoproteins, carbohydrates, fatty acids, peptides and extracellular microbial enzymes such as proteases and pectic enzymes. Host receptors that recognize pathogen elicitors appear to exist outside or on the cell membrane. Once a plant molecule recognises and reacts with a molecule (elicitor) derived from a pathogen, it is assumed that the plant “recognises” the pathogen.

Induced structural defence

CYTOPLASMIC DEFENSE REACTION

The plant cytoplasm surrounds the clump of hyphae and the plant nucleus is stretched to the point where it breaks into two. In some cells, there is no effect and the fungal hyphae growth continues and the protoplast disappears, while in some cells the cytoplasm and nucleus enlarges, cytoplasm becomes granular and dense with the appearance of various particles and structures. Finally, the mycelium of the pathogen disintegrates and the invasion stops.

Cell wall defense structures

They involve morphological changes in the cell wall or changes derived from the cell wall of the cell being invaded by the pathogen. Three main type of structures have been observed in plant diseases:

1. The outer layer of parenchyma cell which comes in contact with the bacteria swells, produce amorphous, fibrillar material which traps the bacteria stop its further growth.
2. Cell wall thickens by producing cellulosic material, infused with phenolics.
3. Callose papillae are deposited (within minutes to 2-3 hours) on the inner side of cell walls in response to invasion by fungal pathogen.

Histological Defense structure

1. Formation of Cork Layers: In plants a several cell layered cork layer is induced beyond the point of infection, as a result of substances secreted by the pathogen. The cork layer inhibit the further invasion and toxic substances secreted by the pathogen beyond the initial lesion. Furthermore, cork layers stop the flow of nutrients and water from the healthy plant to the infected area i.e. to the pathogen. The dead tissues, including the pathogen are thus delimited by the cork layers which may remain there as necrotic tissue or may be sloughed off.
2. Formation of Abscission Layer: It is formed on young leaves between two circular layers of leaf cells surrounding the locus of infection. Upon infection, the middle lamella between these two layers of cells is dissolved throughout the thickness of leaf, completely cutting off the central area of the infection from rest of the leaf. Gradually, this area shrivels, dies and sloughs off, carrying with it the pathogen thus protecting the rest of the leaf tissue from being invaded by the pathogen and its toxic substances.
3. Formation of Tyloses: Tyloses clogs the vessels by forming abundantly and quickly ahead of the pathogen, while the pathogen is still in the young roots, and blocks further advance of the pathogen.
4. Deposition of Gums: Gums are deposited quickly in intercellular spaces and within the cells surrounding the locus of infection, thus forming an impenetrable barrier that completely encloses the pathogen. The pathogen then becomes isolated, starves, and sooner or later dies.

Induced Biochemical Defences

- Plantibodies are antibodies against certain plant pathogens; encoded by animal genes but produced in and by the plant. Eg. – Plantibodies to tobacco mosaic virus in tobacco decreased infectivity of the virus by 90%.
- Resistance through prior exposure to mutants of reduced pathogenicity
- Induction of plant defenses by artificial inoculation with microbes or by treatment with chemicals – Systemic Acquired Resistance (SAR) is characterized by the coordinate induction in uninfected leaves of inoculated plants SAR genes, having antimicrobial activity.
- Defense through genetically engineering disease resistant plants with plant or pathogen derived genes.
- Hypersensitive response

Genetic Control/Mechanism of Plant Immunity

Whatever the kind of defence or resistance a host plant employs against a pathogen, it is ultimately controlled, directly or indirectly, by the genetic material of the host plant and of the pathogen.

It is categorised as –

Nonhost Resistance – To stay resistant (immune) when a plant is brought in contact with a pathogen to which the plant is not a host. Eg. – apple trees are not affected by pathogens of tomato.

Partial/Polygenic/Quantitative or Horizontal Resistance – Depends on many genes for the presence or formation of the various defense structures and for pre-existing or induced production of many substances toxic to the pathogen.

Race-Specific, Monogenic/R gene or Vertical Resistance – The host plant carries one or few resistance genes (R) per pathogen capable of attacking it, while each pathogen carries matching gene for avirulence for each of the R gene of host plant. This host-pathogen combination triggers a defense reaction (hypersensitive response) that neutralizes the pathogen.

2. Fungal toxins as food contaminants

Fungal Toxin or Mycotoxins are toxic fungal metabolites that are released by relatively few but universally present fungi growing on plants, grains, legumes and nuts. Such produce especially when harvested while still containing a high percentage of moisture or if it is damaged or stored at relatively high humidity, becomes moldy, i.e. it supports the growth of mycotoxin producing fungi. During favourable conditions, fungi proliferate into colonies and mycotoxin levels become high in them. Toxins vary greatly in their severity. Some fungi produce severe toxins only at specific levels of moisture, temperature or oxygen in the air. Some toxins are carcinogenic, lethal, some weaken the immune system without producing symptoms specific to that toxin, some act as allergens or irritants, and some have no known effect on humans. Some mycotoxins generally have more negative impacts on farm animal populations than on humans. The diseases caused by the mycotoxins is known as **Mycotoxicoses**. Some mycotoxins are harmful to other micro-organisms such as other fungi or even bacteria; penicillin is one example.

Mycotoxins can appear in the food chain as a result of fungal infection of crops, either by being eaten directly by humans, or by being used as livestock feed. Mycotoxins greatly resist decomposition on being broken down in digestion, so they remain in the food chain in meat and dairy products. Even temperature treatments, such as cooking and freezing, do not destroy mycotoxins.

Various wild mushrooms also contain an assortment of mycotoxins that can cause noteworthy health problems for humans who eat wild mushrooms without first properly identifying the specimens as safe edibles, in such cases sometimes causing mild to catastrophic mushroom poisoning.

Major groups of food toxins in food

Aflatoxins : They are produced by *Aspergillus* species, and are largely associated with commodities produced in the tropics and subtropics, such as groundnuts, other edible nuts, figs, spices and maize. Aflatoxin B₁, the most toxic, is a potent carcinogen and has been associated with liver cancer.

Ochratoxin A : It is produced by *Penicillium verrucosum*, which is generally associated with temperate climates, and *Aspergillus* species which grow in warm humid conditions. *Aspergillus ochraceus* is found as a contaminant of a wide range of commodities including cereals and their products, fruit and a wide range of beverages and spices. *Aspergillus carbonarius* is the other main species associated in warm humid conditions found mainly on vine fruit and dried vine products particularly in the Mediterranean basin. It causes kidney damage in humans and is a potential carcinogen.

Patulin : It is associated with a range of fungal species and is found in moldy fruits, vegetables, cereals and other foods. It is destroyed by alcoholic fermentation and so is not found in alcoholic drinks. It may be carcinogenic and is reported to damage the immune system and nervous systems in animals.

Trichothecenes : *Fusarium* toxins are produced by several species of the genus *Fusarium* which infect the grain of developing cereals such as wheat and maize. They include a range of mycotoxins including the **fumonisin**s, which affect the nervous systems of horses and cause cancer in rodents; and the **trichothecenes**, including deoxynivalenol, and zearalenone, the last two of which are very stable and can survive cooking. The trichothecenes are acutely toxic to humans, causing sickness and diarrhea and potentially death.

Vomitoxin and Zearalenone : They often occur together, especially in scabby wheat and corn infected with *Gibberella*. They have also been found in moldy rice, cotton seed, flour, barley, malt, beer and other foods. In addition to humans they affect cattle, swine, chicken and other birds, cats, dogs and fish. Individuals infected respond by vomiting, refusal to eat, suppression of their immune system, diarrhoea, loss of weight and low milk production in the case of cows.

Fumonisins : Produced by *Fusarium verticilloides* (*F. moniliforme*, *F. proliferatum*) and related species primarily in corn and corn related products. Fumonisin affect all or most of the animals affected by other fusarium toxins but they are particular to horses. In horses even in low concentrations cause liquefaction of the brain, resulting in the “blind staggers” and “crazy horse disease” in which horses display blindness,

head butting and pressing, constant circling and finally die. In swine, it attacks the heart and the respiratory system, in which it causes swellings and it also causes lesions in liver and pancreas. In humans it is linked to cancer.

Mycotoxins killing humans

In 2004 in Kenya 125 people died and nearly 200 others were treated after eating aflatoxin contaminated maize. The deaths were mainly associated with homegrown maize that had not been treated with fungicides or properly dried before storage. Due to food shortages at the time, farmers may have been harvesting maize earlier than normal to prevent thefts from their fields, so that the grain had not fully matured and was more susceptible to infection.

3. Defensins

Defensins are small cysteine-rich cationic proteins found in both vertebrates and invertebrates. They are active against bacteria, fungi and many enveloped and nonenveloped viruses. They consist of 18–45 amino acids. Out of these six to eight amino acids are conserved cysteine residues. Cells of the immune system contain these peptides to assist in killing phagocytized bacteria, for example in neutrophil granulocytes and almost all epithelial cells.

Most defensins function by binding to microbial cell membrane, and once embedded, forming pore-like membrane defects that allow efflux of essential ions and nutrients. The loss of control over membrane transport eventually leads to the death of the microbial cell.

Plant Defensins

Plant defensins are a family of small (~5 kDa) Cys-rich antifungal proteins that play important roles in plant defense against invading fungi. They are small, basic peptides that have a characteristic three-dimensional folding pattern that is stabilized by eight disulfide-linked cysteines. They are termed plant defensins because they are structurally related to defensins found in other types of organism, including humans. Earlier, they are also called as were named gamma-thionins.

Occurrence of Plant Defensins

Nearly all the angiosperm species studied so far display the presence of defensins. Plant defensins are encoded by small multigene families and are expressed in various plant tissues, but are best characterized in seeds. Apart from seeds, leaves, floral organs and the root's zone of differentiation seem to be expressing the defensin genes more actively. Defensins can be classified into four main subtypes according to their specific functions.

Diversity of Plant Defensins

So far, sequences of more than 80 different plant defensin genes from different plant species are available. In *Arabidopsis thaliana*, at least 13 putative plant defensin genes (PDF) are present, encoding 11 different plant defensins. Two additional genes appear to encode plant-defensin fusions.

Structure

Structures of several plant defensins share a Cys-stabilized alpha/beta-motif. As mentioned earlier, there are eight highly conserved cysteine residues linked by disulfide bridges. Interestingly, their structures are remarkably similar to those of insect defensins and scorpion toxins.

In the recent years, the *Brassica* stamen specific plant defensin 1 (BSD1) has been studied in detail by HC Park and co-workers (published in *Plant Molecular Biology*; 2002 Sep). BSD1 transcripts accumulate specifically in the stamen of developing flowers and its level drops as the flowers mature. In BSD1 the eight cysteine residues and a glutamate residue at position 29 are conserved, whereas other amino acid residues of can be substituted without making the defensin lose its activity.

Recent studies by RG Spelbrink and co-workers (2004) shows that the major determinants of antifungal activity in most defensins reside in the carboxy-terminal region (amino acids 31–45). For example, the active site of Alfalfa (*Medicago sativa*) seed defensin, MsDef1 has an Arg at position 38, which is critical for its antifungal activity.

However, Structural determinants in many plant defensins, which govern their antifungal activity, and the mechanisms by which they inhibit fungal growth remain largely unclear.

Activity of Plant Defensins

Plant defensins inhibit the growth of a broad range of fungi but seem nontoxic to either mammalian or plant cells. Plant defensins are generally active against a broad spectrum of fungal and yeast species at

micromolar concentrations. For antifungal activity of defensins, molecular interactions are required between plant defensins and fungi receptors, which include membrane proteins and lipids.

It seems that different plant defensins act by inhibiting or over-stimulating some important membrane transport process. For example, MsDef1, strongly inhibits the growth of *Fusarium graminearum* in by inhibiting Ca^{2+} channels. Some of other defensins interact with fungal-specific lipid components in the plasmamembrane.

Application of Plant Defensins

The transgenic technology has made it possible to transfer defensin gene in susceptible plants with an aim to confer anti-fungal resistance to such plant species. Using molecular techniques it is also possible that the defensin genes may be made to over-express. For example, constitutive over-expression of the BSD1 gene under the control of the cauliflower mosaic virus (CaMV) 35S promoter conferred enhanced tolerance against the *Phytophthora parasitica* in the transgenic tobacco plants.

Defensins seem to have a medical application too. Defensins interact with fungal-specific components in the plasmamembrane, resulting in their membrane permeabilization. This makes them an attractive source of potential therapeutics to treat fungal infections.

4. Jasmonates

Introduction, Chemical Nature and Synthesis

The Jasmonates (Jas) are a group of plant hormones, which regulate plant growth, development and defense responses. Being recently discovered and characterized and also because of being outside the group of classic plant hormones (Auxins, Gibberellins, Cytokinins, Absciscic Acid and Ethylene), they are also called one of "Novel" plant hormones.

The Jasmonates include Jasmonic Acid and its esters, such as Methyl Jasmonate (MeJa). They are cyclopentanone derivatives, and highly similar to the animal hormonal substances Prostaglandins.

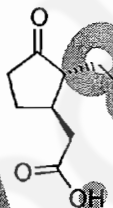


Figure: Jasmonate Structure

They are derived from fatty acids, biosynthesized from linolenic acid (18:3 fatty acid) by the octadecanoid pathway. The important site of Jasmonate synthesis in plants is any green aerial part. The early steps in the biosynthesis are located in the chloroplast, the later steps involving reduction of the cyclopentenone and β -oxidation are performed in the peroxisomes.

The levels of Jasmonates varies widely according to:

1. Function of tissue and cell
2. Developmental stage
3. Different environmental stimuli.

Generally high levels of JA are found in flowers and reproductive structures. Their levels also increase rapidly in response to mechanical disturbances such as tendrils coiling and when plants suffer wounding. Wound induced Jasmonate synthesis is an important defence mechanism against herbivory found in many plant species.

Jasmonate Signaling

The most active Jasmonate compound is MeJa. Plants which are under attack by insects or damaged mechanically produce higher levels of jasmonic acid and methyl jasmonate, which build up in the damaged parts of the plant. Since methyl jasmonate is more volatile than jasmonic acid, it can act as a messenger to neighbouring undamaged plants, signaling them about the attack that is under way. This prompts the neighbouring plants to produce defensive chemicals before they are attacked.

The perception of Jasmonate is via the ubiquitin system, like auxins. It means, proteolysis is involved in Jasmonate signaling. Recent evidences point out that there is destruction of ubiquitin marked JAZ proteins late during Jasmonate signaling. JAZ-1 (jasmonate ZIM-domain) protein in *Arabidopsis thaliana* acts to

repress transcription of jasmonate-responsive genes. Its proteolysis promotes certain type of gene transcription. After this, some other transcription factors are formed which govern jasmonate response in the cell.

Role of Jasmonates in Plant Defense

Methyl Jasmonate (MeJA) is a substance used in plant defense. Plants produce Jasmonic acid and Methyl Jasmonate in response to many biotic and abiotic stresses (particularly herbivory and wounding), which build up in the damaged parts of the plant. Jasmonates act as signaling compounds for the production of phytoalexins. MeJA also stimulates traumatic resin duct production in pine trees. This can be used as a defense against many insect attackers.

Phytoalexins, once ingested by the attacker (e.g., insect), can be toxic or interfere with its digestion and may deter the attacker from further feeding. The Jasmonate signal often spreads systemically throughout the plant and is a major component of systemic acquired resistance.

5. Phytoalexins

An introduction

Phytoalexins are defense substances of low molecular weight with antimicrobial properties produced in plants in response to attack by a pathogen or by chemical or mechanical injury.

In contrast, Phytoanticipins are secondary metabolites produced constitutively.

Allixin (3-hydroxy-5-methoxy-6-methyl-2-pentyl-4H-pyran-4-one), a non-sulfur containing compound having a g-pyrone skeleton structure, was the first compound isolated from garlic as a phytoalexin, a product induced in plants by continuous stress.

More than 300 chemicals with phytoalexin like properties have been isolated from plants belonging to more than 30 families.

They include various groups of natural substances (e.g. isoflavonoids, terpenoids, polyacetylenes and dihydrophenanthrenes).

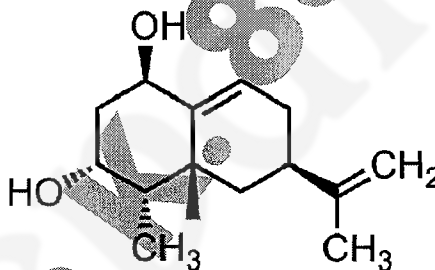


Fig: Capsidiol – a phytoalexin produced by certain plants in response to pathogenic attack.

Special Features

In many systems, Phytoalexin synthesis is strongly enhanced not only upon challenge of plant tissues by parasites but also following treatment with pathogen derived elicitors.

Most phytoalexin elicitors are generally high molecular weight substances that are constituents of the fungal cell wall, such as glucans, chitosan, glycoproteins and polysaccharides.

Action Process

- They are produced by healthy cells adjacent to localized damaged and necrotic cells in response to materials diffusing from the damaged cells.
- Resistance occurs when one or more phytoalexins reach a concentration sufficient to restrict pathogen development.
- Most known phytoalexins are toxic to fungal pathogens, but some are also toxic to bacteria, nematodes and other organisms.

Function

- Act as toxins to the attacking organism.
- May puncture the cell wall, delay maturation, disrupt metabolism or prevent reproduction of the pathogen.

- Involved in the process of conferring long-term or Systemic Acquired resistance (SAR) to plants against pathogen.
- Make plants inedible for vector herbivores.

Caution - Pathogen armed with ability to detoxify phytoalexin

A few fungal enzymes have been found that degrade phytoalexin during fungal attack. One such enzyme is pisatin demethylase, which is produced by the fungus *Nectria haematococca* and degrades the pea phytoalexin pisatin. Pisatin demethylase is encoded by one of six such genes of the fungus.

Concluding remarks

Phytoalexins may play a decisive or an auxiliary role in the defense of some hosts against certain pathogens, but their significance, if any, as factors of disease resistance in most host-pathogen combinations is still unknown.

Chapter 11. Important Plant Diseases

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
RICE			
Blast	<i>Pyricularia</i>	Brown spindle, eye boat shaped lesion on leaf, neck rotting, discoloured nodes, partially filled grains; Heavy dose of nitrogen, high atm- ospheric relative humidity (RH) = 86- 98% and night temp. of 20°C for few hours favour for disease/	Seed treatment with Agrosan GN, Ceresan/ Thiram @ 2g per kg of seeds; spray Zineb or mancozeb @ 0.25%; Grow resistant varieties – Tulsi, Rasi, Swarnadhan; IR-64 Nitrogen management of field.
Bacterial leaf blight (BLB) Or Bacterial leaf spot. (Poor man's disease)	<i>Xanthomonas oryzae</i>	'Kresek' occurs in early stage (Plant withers and dries up); In later stage blighting starts from the tip of the leaves to the base (downward), Straw turned yellow, partially filled grains, Yellowing Bacterial Ooze appears on the surface which dries up mto bead-like incrustations (i.e. Ooze test); Problem under poor and N-deficient soil conditions.	Use disease free certified seeds, water drainage time to time; seeds treatment with Streptomycin 0.015% + ceresin 0.05%; 3-4 sprays of 75g Agrimycin-100 + 500 g copper Oxy chlorides in 500 lit. Water/ha; Hot water treatment; use resistant varieties-Ajay, PR-10; Nitrogen management of field.
Tungro (Leaf Yellowing) BLB & RTV are the Killer disease of Rice)	<i>Rice Tungro Virus (RTV) vector-Rice green leaf hopper</i>	Yellowing from tip & margin on older leaves, stunted growth, empty glumes & poor panicles with dark-brown colouration, Interveinal chlorosis.	Spray systemic insecticide Diazinon @ 1.5 kg a.i. ha; Rogue out diseased plants, slurry treatment of seeds with furadan 75% W.P. @0.13-g/kg seeds; use resistant varieties – Vikramarya.
Brown spot	<i>Cochillobolus miyabeanus (Helminthosporium oryzae)</i>	Many dark- brown elliptical spots on leaves; infects coleoptiles of seedling and causes blighting; infected kernel shriveled.	Seed treatment with ceresin or Agrosan GN @2 g/kg of seeds, grow varieties like Bala, Krishna, Sabarmati, IR-204; Spray dithan M-45 @ 0.25% at 10-12 days interval.
False Smut	<i>Claviceps oryzae sativae (Ustilaginoides virens)</i>	Infected kernels transformed into a large velvety, yellow olive green and more than twice in diameter than normal grains, Infected grains covered with powdery spore mass.	-do-
Khaira disease	<i>Zn-deficiency</i>	Usually in nursery; chlorotic/Yellow patches at leaf base on both sides of midrib; restricted root growth and usually main roots turn brown.	Spray 5 kg ZnSO ₄ + 2.5 kg lime per hectare at 10 DAS in nursery or ZnSO ₄ @ 5 kg + Urea 2% in 1000 litre of water/ha at the sowing time.
Yellow dwarf	<i>Mycoplasma</i>	Minor disease	—
WHEAT			
Rusts			
Brown rust of Leaf rust (LR)	<i>Puccinia recondite tritici</i>	Round oval uredial pustules mainly on leaves and scattered & Irregular; most widespread disease & most	Avoid late sowing use high dose of K, Spray Zineb or mancozeb @0.2% Grow varieties like Sonalika, chhoti

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
		damaging in our country	Lerma, UP-2003, HD- 2285, UP-368, Girija, RH-124; Development of resistant variety through Convergent breeding by using resistant genes viz., Yr, Lr & Sr is being tried.
Yellow rust (YR) or Stripe rust	<i>Puccinia striiformis</i>	Lemon Yellow pustules in rows or long parallels streaks; the pustules of yellow rust are smaller than those of Leaf rust; chiefly on leaves.	—
Black rust or Stem rust (SR)	<i>Puccinia graminis tritici</i>	Elongated uredial pustules on stem, leaf sheath, leaves and earheads but stem is often most severely affected.	—
Kernal bunt or Partial bunt	<i>Neovossia indica</i>	Called cancer of Wheat; grains partly converted into black sooty powder; gives rotten fish smell due to trimethylamine	Since it is soil borne, seed borne & air borne, hence only one solution is to grow resistant variety and seed treatment with mercury fungicide.
Loose smut	<i>Ustilago tritici</i> (<i>Ustilago nuda tritici</i>)	Early emergence of heads; Production of black powder in place of grain; Before ear emergence only Sona lika is detected by yellowing flag leaves & withering; It can be distinguished at ear emergence; Internally Seed-borne. Factors conducive for spread: Wind for spore dispersal; Light rainfall at flowering time; Openness of the flowers; suitable temp around 18-20°C for germination of chlamydospores; Atmosphere should not calm and quiet.	Seed treatment with vitavax @2.5g/kg of seeds, solar heat treatment; Hot water treatment, Raise windbreak plantation to restrict its spread.
Ear Cockle	<i>Arguina tritici</i> (nematode)	Leaf blades generally twisted; infected ears shorter and remain green longer; awns more spreading; affected grains transformed into one or more small galls.	Flotation of Seeds in 20% salt solution; Rouging; use clean seeds.
Tundu disease or Yellow ear rot or Sehun disease	<i>Corynebacterium tritici</i> (bacteria) + <i>Arguina tritici</i> (nematode)	Curling of affected plant leaves; bright yellow slimy ooze (due to bacteria) on leaves and inflorescence; Agglutinated Inflorescence; seeds not formed; grains transformed into small hard galls.	Flotation of Seeds in 20% salt solution; Rouging; use clean seeds.
Molya disease or cereal root eelworm	<i>Heterodera avenae</i> (Cyst nematode)	Stunting, Pale Yellow sparsely growing seedlings; roots showing knots containing nematode cysts.	Crop rotation.

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
BARLEY			
Covered Smut	<i>Ustilago hordei</i>	Smutted head; grains replaced by black agglutinated spore masses & Covered by persistent white papery membrane. Factors for spread – (a) amount of moisture (b) suitable temp. (c) Depth of planting when seeds are planted deep; it take too long time to emerge at the surface. Its dispersal is only at time of harvesting because chlamydospore sticks to seed.	Externally seed borne hence easy to control; Seed dressing with Agrosan GN @2.5g/kg Seeds.
Loose smut	<i>Ustilago nuda</i>	Same as of Wheat	Same as of wheat
Powdery mildew	<i>Erysiphe graminis</i> var. <i>hordei</i>	Cottony growth on both the leaf surface (but on lower surface in downy mildew); later on powdery deposits of conidia; necrosis at powdery spot' Ectophytic; Favourable condition Winter season. Cold and dry weather; control through S fungicides.	Spray 'S' – Fungicide grow resistant variety.
JOWAR			
Downey mildew	<i>Sclerospora sorghi</i>	Downy white growth on lower surface with yellowing on upper surface on young leaves i.e. chlorosis at downy spot; later on shredding of leaf & stunted growth; Endophytic; Favourable condition- Monsoon mainly cold & moist weather	Grow resistant variety. Seed treatment with Agrosan G.N./Ceresan @ 2.5g/kg seed spray maneb or Zineb @0.2%
Grain Smut	<i>Sphacelotheca sorghi</i>	Grains transformed into elongated cylindrical structures consisting of black spore masses.	Seed treatment with finely powdered Sulphur @5g or Agrosan G.N. @2g/kg seed.
Leaf rust	<i>Puccinia purpurea</i>	Bright Purpled spot on leaf surface mainly on lower surface; more severe after flag emergence.	Grow resistant variety like CSH-1, 2 etc; spray Dithan Z-78 @0.2% at 10-12 days interval.
Anthraxnose or Bed leaf spot (BLS)	<i>Colletotrichum graminicola</i>	Brown spots with whitish or purple centres on lower leaves; affects both seedling as well as matured plants.	Grow resistant varieties i.e. CSH-1, CSH-2 etc; Seed treatment with Agrosan G.N.: Spray Zineb @ 0.2%; Weed out Johnson grass (Collateral host)

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
BAJRA			
Downey mildew or Green Ear disease (ERD)	<i>Sclerospora graminicola</i>	Ear transformed into green leaf like or leafy whorl type structure. Essential factor for germination oospores are (1) Good air supply (2) Low soil moisture (3) 20-25°C temp; it means oospore requires weathering; More conducive is Light soil; According to Sateulla & Thirumalachar (1956)- at 15-20°C and 90% RH (Moisture near saturation) sporangia were formed. According to Westson, asexual stage of it is not found in India.	Rouging of diseased Plant, Grow hybrid resistant varieties i.e. HB-3 Spray 0.2% Zineb.
Ergot	<i>Claviceps fusiformis</i>	First appear on the ears in the form of honey like pinkish liquid; Liquid turns brown & sticky; Sclerotia (ergot i.e. pinkish liquid) appears as brown to black later on and elongated structure.	Avoid late planting; Floating of seeds in 2% salt solution, spray Ziram @ 0.15% at boot leaf stage.
Smut	<i>Tolyosporium penicillaria</i>	Affected kernels green & larger in the beginning but later turns to black.	Remove smutted ears; spray vitavax @0.25% Follow three year crop rotation
MAIZE			
Whit but of Maize	Zn-deficiency	Apical portion of leaf becomes white	Apply $ZnSO_4$ @ 20-25 kg/ha at sowing time.
Seed rot or seedling blight	<i>Pythium aphanidermatum</i> ; <i>Fusarium monilliformis</i> ; <i>Rhizoctinia spp</i>		Seed treatment with captan or Thiram @2.0g/kg f seeds.
Black bundle	<i>Cephalosporium acremonium</i>	Decay of Vascular bundle; wilting of leaves & ultimately wilting of plants; black spots in the middle of vascular bundle i.e. black spot on the cut ends of the stalk.	Use resistant hybrids; seed treatment with Bavistin @2g/kg seed (systemic fungicide)
Bacterial Stalk rot	<i>Erwinia crotovorta var zea.</i>	Basal internodes become soft, discolour and starts decaying; alcoholic smell in the field.	No water logging; injury to plant avoided. Rouging of affected plant.
Charcoal rot	<i>Macrophomina phaseoil</i>	Shredding of the pith in the stalk, Black dot on the rind and inside the stalk; lodging of the crops in sever case	Avoid water stress after the flowering of the crop.
GRAM			
Fusarium wilt	<i>Fusarium Oxysporum f.sp. ciceria</i> (4 races)	Stunted growth; yellowing of leaves, withering of plants; Main root turns black	Use of resistant genotypes viz. Puse-212, Phule G-5; Avrodhl; Seed treatment with Bavistin + thiram

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
Sclerotinia blight	<i>Sclerotinia sclerotiorum</i>	Plants become yellow then brown and ultimately dry; All plant parts are affected except roots.	Soil treatment with captan @10 kg/ha; Grow resistant varieties
Ascochyta blight	<i>Ascochyta blight</i> (2 races & 1 biotype)	-do-	Use resistant genotype viz Gaurav; seed treatment with Bavistin & thiram (1:1) or Hexacap @ 3 g/kg seed controls primary infection; spray Dithiaron, @0.1% Indofil M-45; captan @0.2 & Captan @0.2%
ARHAR			
Fusarium wilt	<i>Fusarium oxysporum f. sp. Udum</i>	Most destructive soil borne fungal disease wilting of leaves & plants; lateral roots completely rotten; Tap root become black on the surface & move upward; wilting branches arrive from such blackened area; Black streaks on wood below the bark; disease spread in a circular fashion around the first wilted plant.	No chemical control (i) Follow mixed cropping. Viz Arhar + Tobacco & Arhar + Sorghum. Tobacco liberates chemical injurious to Pathogen & Sorghum's root exudates HCN which is toxic to Fusarium. (ii) Soil amendments with green manuring & use of oil cakes. (iii) Crop rotation with sorghum or tobacco or fallow for 1-2 years.
Sterility mosaic	<i>Sterility mosaic virus (SMV) Vector-Mite (Aceria cajani)</i>	Plants become light greenish bushy; no flowers & fruits	Roughing of Perennial & Self grown plants; only ICPL-151 is resistant in early maturity group; use of acaricide viz Tedion, Morest, Kelthane @1.0% Besides, seed dressing with carbofuran as 25% (Furadan 3G) and seed treatment with 10% Aldicarb or its soil application @1.5 kg.a.i/ ha.
Phytophthora stem blight	<i>Phytophthora drechsleri f.sp. cajani</i>	Serious disease in high humidity and poorly drained soil i.e. state of W.B.; short duration varieties are more susceptible.	Seed treatment with Metalaxyl (1.75g a.i/kg seed) followed by 1 spray of Metalaxyl 25 WP (at 1000 ppm) 30 DAS; proper drainage & Planting on ridges.
Alternaria leaf blight		Serious in post-rainy season	Resistant genotypes are DA-2, DA-11 & Pant A-3.
SOYBEAN			
Yellow Mosaic	<i>Yellow mosaic virus (YMV) Vector-white fly Bemisia tabaci</i>	Yellow mosaic mottling of leaves accompanied with crinkling & reduction in size; stunted plants & few pod setting.	Use resistant variety; spray metastox 25 EC @1 kg/ha in 1000 lit. of water at 10 days interval; Rouging of diseased plants.
Anthraxnose (pod blight)	<i>Colletotrichum truncatum</i>	Pod becomes yellowish & later turns to brown; seed formation seriously affected	Use resistant variety viz Bragg. Spray Zineb @0.25%

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
GROUNDNUT			
Tikka disease & rust or Cercospora leaf spot & rust (CLS)	<i>Phaeoisariopsis personata</i> (<i>Cercospora personata</i> & <i>Cereospora arachidicola</i>) Rust- <i>Puccinia arachidis</i>	Small dark brown spots and Pre-mature leaf shedding. In case of <i>personata</i> , brown spots are regular & not more than 0.6 mm in diameter but of <i>arachidicola</i> , there are irregular spots.	Spray Bavistin @0.05% + Dithan M-45 @0.2% 2-3 times at 2-3 weeks intervals starting from 4-5 weeks after planting.
Collar rot & dry root rot	<i>Aspergillus niger</i> (collar rot)	Attack at seedling stage at the base; black spores are seen at root	Seed treatment with 5g thiram or 3g or Dithane M-45 or 2g Bavistin Per kg kernels; crop rotation
Stem rot	<i>Sclerotium rolfsii</i>		Seed treatment same as of collar rot
RAPE & MUSTARD			
Alternaria blight	<i>Alternaria brassicae</i>	Causes average loss 36%; it causes 10-70% damage while aphid pest causes damage 35-73%; concentric black spots on leaves, stems & pods.	Collect & burn disease debris; remove weeds like coriander; spray captafol (Difoltan; Foltaf) @ 1.5 kg/ha or Dithane M-45 @ 2kg/ 1000 lit. Water at 15 days intervals starting from 40-45 DAS; Hot water treatment of 50°C for 10 minutes of seeds.
White rust. Blister	<i>Albugo candida</i>	White or yellow pustules of variable sizes & shapes on lower surface of leaf; infection covers all parts except roots.	Clean cultivation; remove and burn affected plants; spray zineb @0.2%, captafol (Difoltan, Foltaf) or copper oxyfluoride (Blitox 50 @ 1.5 kg/ha) at 15 days interval.
Downey mildew	<i>Pernospora brassicae</i>	Yellow & irregular spots on upper surface & white growth on under surface of leaf; malformed inflorescence	Spray 0.2% Zineb at 10 days interval.
SUNFLOWER			
Alternaria blight or Leaf spot	<i>Alternaria helianthi</i>	Small oval spots on leaves	Spray Mancozeb (Indofil M-45) Zineb (Indofil Z- 78) @0.25%.
Rust	<i>Puccinia helianthi</i>	—	Seed treatment with Brassical followed by Thiram and Mancozeb
Root & collar rot	<i>Puccinia helianthi</i>	—	Seed treatment with Thiram and Mancozeb
Downey mildew	<i>Plasmopara halatedil</i>	—	Seed treatment with APRO 35 SD (metaloxyl Comp) @ 6g/kg seed
COTTON			
Bacterial blight	<i>Xanthomonas</i>	Major disease of cotton; angular and water soaked lesions on leaf & stem.	Spray Streptocycline + copper oxychloride; seed treatment with agrimycin 100; destroy debris.
Fusarium wilt	<i>F. Monilliform f sp. Vasinfectum</i>	Vascular tissue becomes brown; only scattered plant affected	Grow resistant variety like American cotton; apply K + O.M.
Anthracnose	<i>Colletorichum indicum</i>	Dark brown spots on the stem below soil surface and on roots; circular &	Seed treatment with ceresin/Agrosan G.N. ; spray Blitox

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
		water soaked spots on bracts and spread to bolls.	
Myrothecium leaf spot	<i>Myrothecium roridum</i>	—	Spray carbendazim 0.1% or copper oxychloride (0.2%)
Tirak	<i>Physiological</i>	Premature defective openings of bolls shedding of leaves	Late sowing, apply extra water at flowering & fruiting in sandy soil.
Root rot/Dry root rot/Sore shin	<i>Rhizoctonia bataticola</i> <i>Macrophomina phaseoli</i> <i>Rhizoctonia solani</i>	—	Mixed cropping with Moth (<i>Phasaolus aconitifolium</i>)
Grey mildew or Dahiya disease	<i>Ranularia areola</i>	Serious only in desi cotton; cloudy weather followed by rains and temp. 24-26°C favours this disease.	Dusting of 's' or spray 0.1% carbendazim or 0.2% kalthane.
Stenosis (small leaf)	<i>Virus</i>	Extreme stunting of the aerial organs.	
SUGARCANE			
Red rot	<i>Collectorichum falcatum</i>	Red rot inside the stalk; red area traversed by white band; alcoholic smell from field.	Setts treatment with 0.25% Agallol or Aretan (1:100) solution; avoid rationing; use healthy seeds; Heat treatment.
Grassy shoot disease (GSD) or (Albino)	<i>Mycoplasma</i>	Excessive tillering, sprouting of lateral buds	Use healthy setts, Hot water treatment or Most hot-air therapy or aerated stem (15°C for 1 hr.)
Ratoon Stunting disease (RSD)	<i>Ratoon stunting virus (RSV)</i>		Heat treatment, application of 'S'
POTATO			
Late blight	<i>Phytophthora infestans</i>	Bright brown & irregular patches starts from leaf tip or leaf margin; later on turned in brownish black patches; Ground leaves show symptom first; in favourable condition entire vegetative parts are killed within a day hence called blight; in unfavourable environment infected area becomes brittle & detached; continuous high humidity and relatively low temp are favourable condition. In Indo Gangetic plains only mode of survival is infected tubers stored at low temp.	Healthy seed material, high ridging to reduce infection, delayed harvesting because of high temp & the above parts is dried away. Due to labour intensive Bordeaux mixture is not used; Blitox 50 is also not used; spray Maneoze/Zineb @ 2.0 – 2.5 kg. 10 lit. of water/ha; 2-3 sprays at 15 days interval; tuber treatment with mercuric chloride (1:1000 in water); Use resistant variety like kufri Alankar, kufri jyoti & Kufri chandramukhi developed by CPRI, Shimla.
Early blight	<i>Alternaria solani</i>	Concentric ring or target board appeared on leaf lamina; spots irregularly distributed; No any particular favourable condition	Spray Zineb/Maneb @0.2% Sort out infected tubers.

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
		hence it is widely distributed; collateral host is Tomato.	
Black scurf	<i>Pellicularia filamentosa</i> (<i>Rhizoctonia solani</i>)	Surface of tuber covered with black incrustations (Sclerotia of pathogen), growing tips of tuber sprouts are killed.	Tuber treatment with mercury chloride (1: 1000); soil treatment with Brassicol @20-30 kg/ha as furrow application.
Wart	<i>Synchytrium endobioticum</i>	Appearance of tumours or warts on tubers, stems & stolons.	Grow resistant variety.
Charcoal rot	<i>Macrophomina phasei</i>	The roots become brown & rot; the bark of stem becomes ash coloured; black sunken areas are formed around the stem end of the tuber.	Early harvesting; tuber treatment with Agallor/Aretan grow resistant variety.
TOBACCO			
Tobacco mosaic	Virus	Mosaic mottling, blistering & puckering of young developing leaves.	Strict sanitary measures; spray 1% tannin acid; Rouging.
Damping off	<i>Pythium aphanidermaum</i>	Seedling rot at collar-region	Pre-seeding application of Metalaxyl MZ @2.16 kg/ha followed by 2-3 post-emergence application.
Tobacco Leaf curl (TLC)	<i>Tobacco leaf curl virus (TLCV)</i> Vector: whitefly	Okra & castor support the growth of vector but are non-hosts of TLCV while cluster bean, Sesame, sunhemp & chillies are alternative hosts of TLCV.	Clean the alternative hosts & other weeds.
TOMATO			
Tobacco mosaic	Virus	Same as of tobacco	
Late blight		Same as of Potato	
Early blight		Same as of Potato	
Leaf curl	Virus	Curling of leaves, thick & leathery leaf	Spray systemic insecticide Dimethoate 0.1% at one week interval before fruit ripening.
Blossom end rot (Buck eye rot)	Physiological due to Ca deficiency, uneven moisture supply	Black, sunken, necrotic lesions, Water-soaked as the blossom end of green or ripening fruits.	Foliar spray of CaSO ₄ 0.40.5% avoids irregularity in moisture supply; spray captafol 0.3% at 10 day interval.
Cracking	Physiological due to B- deficiency of heavy rain/irrigation after a long dry spell	Radial and concentric cracking at the upper side of fruit.	Spray Borax @0.2% in soil @ 15.20 kg/.ha.
APPLE			
Scab	<i>Venturia inaequalis</i>	Scattered, circular brown spots with dendritic margin on undersurface of leaves; dark brown spots on fruits.	Pre-blossom spraying with lime sulphur (1:60) or Benomyl at fortnightly interval.

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
Bitter rot	<i>Glomerella cingulata</i>	White powdery growth	Remove affected fruits; apply Bordeaux mixture (4 : 5 : 50).
Powdery mildew	<i>Podosphaera leucotricha</i>	Rotting of premature fruits & continue upto storage	Spray Bordeaux mixture, Karathane E.C. (0.05% or carbendazim (0.05%).
Black canker	<i>Sphaeropsis malourm</i>	Cankorous, elongated corky lesion on stem; bark cracks, peels off	Only control is Pruning
MANGO			
Malformation	<i>Fusarium monilliformae</i> var. <i>subglutinana</i> (Previously considered mite, Virus & Physiological)	Vegetative and floral malformation bunchy top appearance; more around cities or settlement than in open country-side due to particulate type of pollution.	Pruning; spray captan; single spray of NAA or planofix in the conc of 200 ppm by deblossoming at bud burst stage.
Anthraxnose	<i>Colletotrichum gloeosporioides</i>	Dark brown spot on leaves	Spray 1% Bordeaux mixture of Metalaxyl
PAPAYA			
Ring spot or Papaya ring spot or Distortion ring spot	<i>Ringspot virus (RSV) Papaya ringspot virus (PRSV) Distortion ringspot virus (DRSV)</i> Transmission by mechanical sap. Inoculation and aphid vectors.	ELISA test confirmed that the mosaic virus of India belongs to papaya ringspot virus (PRSV); PRSV is serious disease, infected leaf rolls upwards along the margins distinct round spots on main stem which turns into elongated streaks on leaf petiole & upper half of the stem; malformation in winter; yellow spots & yellow rings with solid green center on matured green fruit.	No resistant variety to PRSV. Spray malathion to control aphid vector (<i>Aphis gossypii</i> A. <i>cracivora</i> , <i>Myzus persicae</i>)
Leaf curl	<i>Viral</i>	Curling, crinkling and distortion of leaf.	Uprooting of infected plants at early stage.
Papaya mosaic	<i>Viral</i>	Faint chlorotic spots on leaf surface followed by vein clearing, pickling & mottling of young leaves; in extreme case leaf blade distorted & modified into shoe string; identical to Ringspot.	Same as of ringspot
Stem & Foot (Collar) rot	<i>Pythium aphanidermatum</i>	Water soaked patches & swollen collar of the stem.	Good drainage & spray 1% Bordeaux mixture (BM)
BANANA			
Panama wilt	<i>Fusarium oxysporum varcubense</i>	Progressive browning & falling of leaves. Black streaks on underground stem. Serious on Rasthali group of banana & Cavendish group is not susceptible.	Use disease free suckers; crop rotation; eradicate affected plants.
Bunchy top	<i>Virus (BBTV) Banana Bunchy top virus Vector. Pentalonia</i>	Leaves shot & narrow; bunched together at top	Use virus free sucker; rouging.

Crops and Diseases	Pathogen	Symptoms & Feature	Control measures
	<i>nigronevoga</i>		
Konkan disease or Banana bract mosaic	Viral (Banana bract mosaic virus – BBMV)	Disease of Nendran banana in Kerala; spindle shaped pattern in unusually red colored pseudostem; dark streak on petiole base) reddish streaks on bracts & undersized fruit.	Banana Streak disease
Banana Steak disease	Banana Steak virus (BSV) Vector : Mealy bug (<i>Planococcus citri</i>)		
Sigatoka or Leaf spot	<i>Mycopharella musicola</i>	Prevalent in humid tropics or coastal regions.	
CITRUS			
Citrus Canker	<i>Xanthomonas compestris pvcitri</i>	Most serious disease of acid lime; small brown raised corky outgrowths on leaves, twigs, fruits	Prune & burn; use only disease free planting material spray 1% B.M.
Die-back or wither tip or twig blight	<i>Collectotrichum gloeosporioides</i> <i>Diplodia natelansis</i> <i>Fusarium sp.</i>	Dieback of young twigs; black dots on dead tissues	Prune & Bordeaux paste paint to cut ends; spray with Zn-Cu lime.
Gummosis (Broom rot)	<i>Phytophthora palmivora</i>	Ruptured lengthwise bark, exudes gum	Spray 0.1 aurofungin
Greening	<i>Gracillicuts gram</i> negative bacteria (previously considered mycoplasma) Vector- <i>Diaphorina citri</i>	Yellowing of midribs & lateral veins of leaves;	Drenching with tetracycline; spray B.P. 101 (500 ppm)
Tristeza	<i>Citrus tristeza virus</i> (CTV) vector- <i>Toxoptera citricida</i>	Gradual decline in vigour	Use resistant root stock viz. Rangpur lime.
Decline/chlorosis	Insufficient soil moisture and nutrition	Yellowing and gradual reduction in the size of the leaves; die back of the twigs followed by decline and gradual death	-do-
Root rot	<i>Phytophthora palmivora</i> <i>Phytophthora citrophthora</i> <i>Phytophthora parasitica</i> <i>Phytophthora nicotianae</i> var. <i>parasitica</i>	Use of tolerant root stock; Drenching with Ridomil and Foltaf.	-do-

Chapter 12. Integrated Plant Disease Management (IPDM)

Integrated Plant Disease Management (IPDM)

IPDM involve management systems which utilize compatible combinations of all the available techniques to keep the pathogen population below the economic threshold level (ETL) which would not result in economically unacceptable damage to the crop. IPDM is based on five principles of plant disease management and integrates multidisciplinary approaches for the management of plant diseases.

Main components of IPDM

1. Cultural practices
2. Regulatory measures (quarantine)
3. Chemical methods
4. Biological methods
5. Physical methods
6. Genetic engineering

Main strategies of IPDM

1. Need based application of pesticides
2. Encouragement and enhancement of biocontrol agents
3. Use of resistant or tolerant cultivars of plants
4. Modification of cultural practices
5. Use of any other strategies that interrupts host-pathogen interactions

Advantages of IPDM

1. Avoids chemical pollution of soil, water, air and food products
2. Avoids development of resistance in the plant pathogens against fungicides
3. It is an eco-friendly strategy for management of plant diseases
4. It is an economically feasible approach
5. It is a multipronged strategy for efficient management of plant diseases

Therefore, IPDM utilizes all suitable strategies in a compatible manner to reduce and maintain pathogen populations at levels below those causing economic losses.

Rice diseases and IPDM

Fungal diseases

1. Blast: Foliar disease and the pathogen survives on collateral hosts
2. Brown spot of rice – Seed borne and a foliar disease
3. Sheath rot, sheath blight, foot rot and stem rot – Soil borne diseases
4. False smut – seed borne disease

Bacterial diseases: Bacterial leaf blight and bacterial leaf streak – Seed borne and survives on collateral hosts and weeds

Viral or Phytoplasmal diseases – Rice tungro virus, Rice yellow dwarf – Survives on weeds and dissemination is by insect vectors

IPDM strategy in rice

1. Selection of healthy seed
2. Selection of resistant cultivars
3. Removal and destruction of collateral hosts
4. Balanced fertilization
5. Rouging of diseased plants
6. Seed treatment with carbendazim or tricyclazole at 2g/Kg seed
7. Need based foliar application of carbendazim@0.1% or Tricyclazole@0.06% for the management of blast.
8. Need based foliar application of validamycin for the management of sheath blight and sheath rot.
9. Soil application of carbofuran granules or foliar spray of any systemic fungicide is followed to manage insect vectors, thereby decreasing the spread of viral diseases.

Sugarcane diseases and IPDM

1. Red rot – sett borne disease which spreads through irrigation water
2. Whip smut – sett borne and disseminate through wind borne sporidia
3. Pine apple disease, sett rot – Sett borne disease
4. Grassy shoot – Vector borne Phytoplasmal disease
5. Ratoon stunting – Sett borne (*Clavibacter xyli*)
6. Sugarcane mosaic – Survives on weeds and disseminated by insect vectors

IPDM in sugarcane

1. Collection and destruction of infected crop debris
2. Hot water treatment of setts (52°C for 30 min)
3. Hot air treatment of setts (54°C for 2-3 hrs)
4. Balanced irrigation and fertilization
5. Avoid selection of seed material from Ratoon crop
6. Need based spray of systemic insecticides to minimize the spread of viral and Phytoplasmal diseases
7. Selection of disease resistant or tolerant cultivars

Biotechnology: It is defined as genetic modification and manipulation of living organisms through the novel technologies such as tissue culture and genetic engineering resulting in production of improved or new organisms that can be used in variety of ways.

Application of Biotechnology in Plant Disease Management

1. Diagnosis of plant diseases

a) Diagnostic kits helps in identification of plant diseases, viz., bacterial canker of tomato, soybean root rot, viral diseases of potato, etc., at an early stage of development and helps in devising suitable management practices.

b) Polymerase Chain Reaction (PCR): Detection of very small amount of pathogen in a sample by amplifying the pathogen sequences to a detectable level. PCR is especially used in plant quarantine.

2. Strain improvement of biocontrol agents: It has the following advantages

- a) Expanding the range of target species
- b) Restricting the range of non-target species
- c) To improve the survival ability or rhizosphere competence
- d) Expanding the bio-agents environmental range beyond its congenial habitat
- e) Development of fungicide tolerant strains

3. Transgenics for plant disease management

- a) Coat protein mediated resistance for papaya ring spot virus in Hawaii islands
- b) Cloning of resistance genes, viz., Xa 21, bacterial blight resistance gene isolated from African rice, *Oryza longistaminata* was introduced into cultivable rice, *Oryza sativa*

4. Determination of biochemical nature and the signals involved in plants reaction to pathogen invasion and disease development. Ex: Host-pathogen interaction has been studied in rice blast disease incited by *Magnaporthe grisea*.

5. Manipulation of resistance of host by expression of PR-proteins, antifungal peptides, etc. Ex: Expression of multiple PR-proteins (Chitinases and β -1,3 glucanases) in rice enhanced disease resistance to rice sheath blight pathogen, *Rhizoctonia solani*.

Plant Tissue Culture: *In vitro* culture of plant cells, tissues as well as organs

Totipotency is the ability of a plant cell to perform all the functions of development which are characteristic of zygote, i.e., its ability to develop in to a complete plant.

Important tissue culture techniques of importance to plant pathology:

- 1. Meristem tip culture
- 2. Protoplast culture

1. Meristem tip culture: Cultivation of axillary or apical meristems, particularly of shoot apical meristem, is known as meristem culture.

- **Explant:** the explant must consist of the meristematic dome of cells together with atleast one leaf primordial. Meristem tips varying in size from 0.1 to 2.0 mm in diameter (usually 0.3-1.5 mm) can be used for meristem tip culture. The infected parent plant or organ of the plant from which explant is excised is generally subjected to thermotherapy in a temperature controlled cabinet at 30°C to 40°C for six to twelve weeks to inactivate the virus.
- **Culture initiation on suitable medium:** In general Murashige and Skoog medium has been found satisfactory for most plant species. But for some species, a much lower salt concentration may be adequate or even necessary since the high salt concentration of MS medium may be deleterious or even toxic. Culture initiation consists of surface sterilization of explants and establishing them *in vitro* on culture medium. Culture initiation often involves anti-metabolite chemicals such as ribavirin (virazole) in the tissue culture medium.
- **Shoot multiplication:** After 2-3 weeks, the cultures are transferred to a shoot multiplication medium designed to promote axillary branching. This medium generally contains

cytokinins, either alone or in combination with an auxin. Higher concentration of cytokinins induces adventitious buds. During culture initiation and shoot multiplication phases, the cultures are generally kept at 25°C.

- **Rooting of shoots:** In general, the rooting medium has low salt (1/2 or even 1/4 salts of MS medium) and reduced sugar levels. But in most species, 0.1–1 mg/l Naphthalene Acetic Acid (NAA) or Indole-3-Butyric acid (IBA) is required for rooting. Rooting takes about 10–15 days depending on species.
- **Transfer of plantlets to soil:** Rooted shoots are removed from the medium, agar sticking to roots is washed with tap water, and they are transplanted into plastic cups containing a suitable potting mix. Plants are kept in high (>90%) humidity and initially low light intensities. The humidity is generally decreased to the ambient level after about 7–15 days, and the light intensity is increased. The plants are finally exposed to greenhouse conditions (**hardening**).
- **Indexing, clone selection and stock maintenance:** Virus indexing is done several times during first year and the virus free plantlet is used as a nuclear stock material for commercial multiplication. Virus indexing is generally made by Enzyme Linked Immuno-Sorbent Assay (ELISA) or Immuno Sorbent Electron Microscopy (ISEM).

2. Protoplast culture: Fungal protoplasts are important tools in physiological and genetic research. Interspecific, intraspecific and intragenetic hybridization could be done by this technique for strain improvement of biocontrol agents to enhance the biocontrol potential for the management of pathogenic fungi. Isolation and self-fusion of protoplasts were achieved in *Trichoderma harzianum* and *T. viride*.

Steps in protoplast fusion:

- Isolation of protoplasts is achieved by treating cells with a suitable mixture of cell wall degrading enzymes.
- The pH of enzyme solution is adjusted between 4.7 and 6.0 and temperature is kept around 25–30°C. The osmotic concentration of enzyme mixture and of subsequent media is elevated to stabilize the protoplasts and to prevent them from bursting. Usually, 50–100 m mol/l CaCl₂ is added to the osmoticum as it improves plasma membrane stability.
- The protoplasts of different strains are treated with 28–50% Poly Ethylene Glycol (fusogen) for 15–30 min followed by gradual washing of the protoplasts to remove PEG. The washing medium may be alkaline and contain high calcium ion concentration (50 m mol/l). Protoplast fusion occurs during washing step.
- Selection of hybrid cells and culturing on suitable medium.

Gene cloning/ Recombinant DNA technology / Genetic engineering

Integration of specific fragment of foreign DNA into a cell through a suitable vector in such a way that the inserted DNA replicate independently and transferred to progenies as a result of cell division.

Recombinant DNA molecule is a vector into which the desired DNA fragment has been inserted to enable its cloning in an appropriate host. Recombinant DNA molecule is produced by joining together two or more DNA segments usually originated from different organisms.

Steps in gene cloning

1. Identification and isolation of the desired gene or DNA fragment to be cloned (Restriction digestion and electrophoresis)
2. Insertion of the isolated gene in a suitable vector (ligation)
3. Introduction of this vector into a suitable organism or cell called host (transformation)
4. Selection of transformed host cells (selectable markers)
5. Multiplication / integration followed by expression of the introduced gene in the host

Enzymes involved: Restriction endonucleases, DNA ligases, DNA polymerases, RNA polymerases and reverse transcriptases.

Vectors used in gene cloning: A vector is a DNA molecule that has the ability to replicate in an appropriate host cell, and into which the DNA fragment to be cloned (called DNA insert) is integrated for cloning. Ex: Tumor inducing (Ti) plasmid of *Agrobacterium tumefaciens*, pBR322, Bacteriophages, cosmid vectors (derived from phage λ).

Ti plasmid of *Agrobacterium tumefaciens*

- Ti plasmid is a large conjugative plasmid or megaplasmid of about 200 kb.
- Ti plasmid has a T-DNA region (15–24 kb) which is bounded by a pair of 24 bp repeats. T-DNA carries genes for **auxin**, **cytokinins** and **opine** synthesis which are responsible for tumor formation (tumorigenesis).
- Transfer of T-DNA depends on 35 kb **virulence (vir) region** of the Ti plasmid. This region has 7 operons ranging from *vir A* to *vir H* (*vir A*, *vir B*, *vir C*, *vir D*, *vir E*, *vir G* and *vir H*). The protein products of these genes respond to phenolics to generate a copy of T-DNA and mediate its transfer into the cell.
- The T-DNA when transferred from the *Agrobacterium* to the plant cell integrates with the chromosome, and the plant cells which are affected begin to synthesize opines, auxins and cytokinins.
- Opines are tumor specific compounds formed by the condensation of amino acid, keto acid and sugar. The **opines** (**octopine**, **nopaline**, **succinamopine** or **leucinopine**) can be metabolized only by *Agrobacteria*.
- The IAA (auxin) and Isopentenyl-AMP (cytokinins) are phytohormones which cause the proliferation of plant cells and induction of the gall.
- Plant wound exudates contain phenolics, which attract *Agrobacterium* and induce *vir* genes. The strong *vir* gene inducers are syringic acid, ferulic acid, **acetosyringone** and sinapinic acid. Only *Agrobacterium* with Ti plasmid are attracted by these compounds.
- The exogenous DNA is inserted into the T-DNA region of the Ti plasmid by homologous recombination using an intermediate vector system or directly using binary vectors.

Development of disease resistant transgenic plants through Ti-plasmid mediated gene transfer:

- The appropriate gene construct is inserted within the T-DNA of a disarmed Ti plasmid; either a co-integrate or binary vector is used. The recombinant vector is placed in *Agrobacterium*, which is co-cultured with the plant cells or tissues to be transformed for about 2 days.
- In case of many plant species, small (a few mm diameters) leaf discs are excised from surface sterilized leaves and used for co-cultivation. In general the transgene construct involves a selectable reporter gene (Bacterial *neo* gene), the presence of which confers resistance to kanamycin.
- During the leaf disc-*Agrobacterium* co-culture, **acetosyringone** released by plant cells induces the *vir* genes which bring about the transfer of recombinant T-DNA into many of the plant cells. The T-DNA would become integrated into the plant genome, and the transgene would be expressed. As a result, the transformed plant cells would become resistant to kanamycin.
- After 2 days, the leaf discs are transferred onto a regeneration medium containing appropriate concentrations of kanamycin and carbenicillin. **Kanamycin** allows only transformed plant cells to divide and regenerate shoots in about 3–4 weeks, while

carbenicilin kills *Agrobacterium* cells. The shoots are separated, rooted and finally transferred into soil.

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PART – IV

ALGAE

Introduction to Algae

What are Algae?

The term Algae is a non-taxonomic expression in modern Botany as it covers a polyphyletic and paraphyletic group of organisms. The algae include all oxygenic photosynthetic organisms excepting plants. Plants are defined as oxygenic photosynthetic organisms which form embryo from zygote, and they include Mosses, Liverworts, Hornworts, Ferns and allies, Club mosses, Conifers, Cycads, Ginkgos, Gnetophytes and flowering plants.

The algae are oxygenic photosynthetic organisms but they do not form embryo from zygote. Further, their sex organs are naked – i.e. without any jacket – and reproductive processes inconspicuous, a reason for which they have been grouped under Cryptogams. Such a definition of algae allows inclusion of oxygenic photosynthetic prokaryotes such as the cyanobacteria plus the photosynthetic eukaryotes which do not form embryo, such as green algae, brown algae, red algae etc.

The algae are primarily aquatic organisms. They are dominating primary producers in aquatic ecosystems, on unstable substrates (muds) and in intertidal marine habitats.

Algae are distinguished from each other on a number of different characteristics. The most important ones are:

1. The combination of photosynthetic pigments.
2. The presence of flagella and if so how many, how do they insert in the cell and how do they beat?
3. Is the cell surrounded by extracellular material? If so, what is that material—organic or inorganic, a continuous wall or a layer of scales?
4. Are the cells motile or not?
5. Do they occur singly, in colonies, filaments or exhibit differentiation to satisfy the criterion of multicellularity?

Evolutionary lineages of algae

According to the Tree of Life Project, there are 8 evolutionary lineages to which the algae belong. However, it is important to note that all members of these lineages are not regarded algae.

These eight lineages are summarised below.

Group	Composition	Organization	Major pigments
<i>Alveolates</i>	Contains some algae, autotrophic Dinoflagellates, diverse, Peridinium, Symbiodinium, Ceratium	Unicellular, Colonial, Syncytial; Free-Living, Symbiotic And Parasitic	chlorophylls a and c, some symbionts
<i>Chlorarachniophytes</i>	A few genera of amoeboid organisms all with symbiotic Chlorarachnion	Syncytial, Free-Living	Chlorophyll b
<i>Cryptomonads</i>	About 12 genera of flagellates, Cryptomonas	Single Cells, Rarely Forming Colonies, Some Are Endobiotic	Chlorophylls a and c, phycobilins
<i>Euglenids</i>	about half of the genera (35) contain members with green chloroplasts, flagellates, Euglena, Trachelomonas	Single Cells	Chlorophyll b
<i>Glaucophytes</i>	Several genera of flagellated and non-flagellated protists with similar phycobilin-rich symbionts, e.g.	Flagellated And Non-Flagellated Cells	Phycobillin

	Glaucocestis, Cyanophora		
<i>Haptophytes</i>	Diverse, with many genera, all or all bar one genera with plastids, with naked species and those with scales (coccolithophores)	Single Cells, Some Are Endosymbionts	Chlorophylls a and c
<i>Red algae (Rhodophyta)</i>	All species are regarded as algal	Free-Living And Parasitic, Single Celled, And Multicellular	Phycobilins
<i>Stramenopiles</i>	Most but not all stramenopiles are algae, the group includes diatoms, brown algae, Synurophytes and other 'Chrysophytes'	Single Celled, Colonial And Multicellular, Free-Living And Parasitic	Chlorophylls a and c
<i>Viridaplantae</i>	The green algae, all but a few genera are algal, Prasinophytes, Chlorophyta (e.g. Volvocalean Algae, Conjugatophytes, Ulvales, Charales)	Single Celled, Colonial And Multicellular, Free-Living	Chlorophyll b

Unifying features of algae

- Algae are a very large and diverse group of simple organisms.
- Their habitat is primarily aquatic but other locations such as mud, snow, bark of trees, moist soil are also occupied by algae.
- Except a few parasitic forms such as *Cephaleuros*, all algae are autotrophic organisms. Some groups, however, contain members that are mixotrophic, deriving energy both from photosynthesis and uptake of organic carbon either by osmotrophy or phagotrophy.
- They show a great diversity in structure—ranging from unicellular to multicellular forms, such as the giant kelps that grow to 65 meters in length. The prokaryotic cyanobacteria are also referred to as blue-green algae.
- The algal somatic structure is simple and called a thallus because it lacks most of the distinct tissue and organ types found in land plants. A range of algal morphologies are exhibited. Most of the simpler algae are unicellular flagellates or amoeboids, but colonial and non-motile forms are also found in several of the groups. Some of the more common organizational levels are:
 - Colonial:** Small, regular groups of motile cells
 - Capsoid:** Individual non-motile cells embedded in mucilage
 - Cocoid:** Individual non-motile cells with cell walls
 - Palmelloid:** Non-motile cells embedded in mucilage
 - Filamentous:** A string of non-motile cells connected together, sometimes branching
 - Parenchymatous:** Cells forming a thallus with partial differentiation of tissues

In three lines even higher levels of organization have been reached, with full tissue differentiation. These are the brown algae, some of which may reach 50 m in length (kelps)—the red algae, and the green algae. The most complex forms are found among some of the green algae (Charales). The Charales represent a lineage that eventually led to the evolution of higher land plants.

- Algae exhibit a wide range of reproductive strategies, from simple, asexual cell division to complex forms of sexual reproduction. In algae reproduction takes place in 3 ways: 1. Vegetative 2. Asexual and 3. Sexual.

Vegetative Reproduction

In some unicellular organisms reproduction is by cell division. The divisions may be repeated in rapid succession. This is called binary fission (Fig.1A).

In colonial and certain multicellular algae, cell division and subsequent enlargement occur. Many filamentous forms, non-colonial and other multicellular algae reproduce by fragmentation. The fragments

have the capacity to continue growth and develop into new individuals. Among the filamentous blue-green algae this is a specialised process and the fragments which exhibit gliding movement are called hormogonia (Fig.1B).

In coenobial algae the reproduction is by autocolony formation. Autocolony is a miniature colony produced by a cell of a parent colony (Fig.1C).

Algae produce a variety of spores out of which akinetes are very common in blue-green and green algae. An akinete is a vegetative cell with its wall thickened and can withstand the unfavourable conditions (Fig.1D).

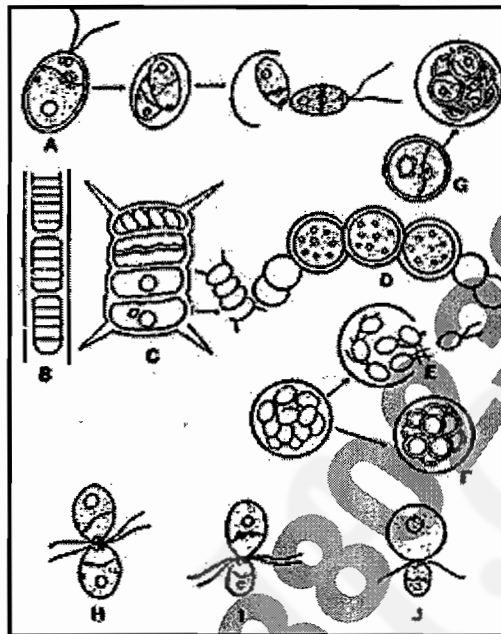


Figure 2: Methods of reproduction (Diagrammatic) A. Bipartition or binary fission. B. Fragmentation or hormogone formation. C. Autocolony formation. D. Akinete formation. E. Zoospore formation. F. Aplanospore formation. G. Autospore formation. H. Isogamy. I. Anisogamy. J. Oogamy.

Asexual Reproduction

Asexual reproduction is achieved by the production of various types of spores. Except Cyanophyceae and Rhodophyceae, most of the groups produce zoospores which are motile unicells (Fig.1E). In some cases the zoospores lose their motility and such spores are called aplanospores (Fig.1F). In some, the aplanospores appear identical to the parent cell and these are referred to as autospores (e.g., *Chlorella*, Fig.1G). Sometimes the aplanospores thicken their walls and develop into hypnospores. The endospores and exospores of Cyanophyceae, monospores, tetraspores etc. of red algae are other types of asexual spores. These are described in detail under the individual groups. In reproduction the word 'swarmer' is commonly used for a motile spore which behaves either as a zoospore or as a gamete. In multicellular forms the spores may be formed in all cells or it may be restricted to well defined 'sporangia'.

Sexual Reproduction

In sexual reproduction there is an opportunity for exchange of genetic material and formation of new combinations. Sexual reproduction is not observed in Cyanophyceae. It is not yet confirmed in Euglenophyceae. In all the other classes of algae it is present. This is affected by three basic methods: 1. Isogamy 2. Anisogamy and 3. Oogamy. In isogamy fusion occurs between two morphologically identical gametes. Anisogamy involves the pairing of two dissimilar gametes, i.e., one gamete is smaller than the other. In certain cases the morphologically identical gametes behave differently, thus exhibit the physiological anisogamy. In some algae the gametes may be highly dimorphic. The larger, non motile gamete is called an egg or ovum and the smaller, motile one is called the sperm or spermatozoid. A spermatozoid unites with an egg and this type of sexual reproduction is known as Oogamy (Fig.1H-J). After the fusion of the gametes a zygote is formed. The germination of zygote may vary in different algae but usually the contents divide to form zoospores. These zoospores germinate into the parent plant. In rare cases the zygote germinates directly into an adult plant. In Charophyceae the germination of the zygote is indirect and produces a protonema from which the adult plant develops.

Different types of life-cycles

Different types of life-cycles have been recognised in the algae:

1. **Haplontic:** In this, the parent is haploid and the zygote represents the diploid phase with reduction division occurring at the time of the germination of the zygote (e.g., *Volvox*, *Oedogonium*).
2. **Diplontic:** The parent is a diplont and the sexual spores (gametes) constitute the haploid phase. Reduction division takes place at the time of gametogenesis (e.g., Diatoms).
3. **Diplo-haplontic:** There will be an alternation of diploid sporophyte with the haploid gametophyte. Reduction division is conducted at the time of formation of spores by the sporophyte (e.g., *Cladophora*).
4. **Haplo-biontic:** Two haploid generations (gametophyte and carposporophyte) alternating with a diploid one represented by the zygote (e.g., *Batrachospermum*).
5. **Diplo-biontic:** Two diploid phases (carposporophyte and tetrasporophyte) and a haploid phase (gametophyte) alternating with each other (e.g., *Polysiphonia*).

A detailed account of various types of algal life cycles

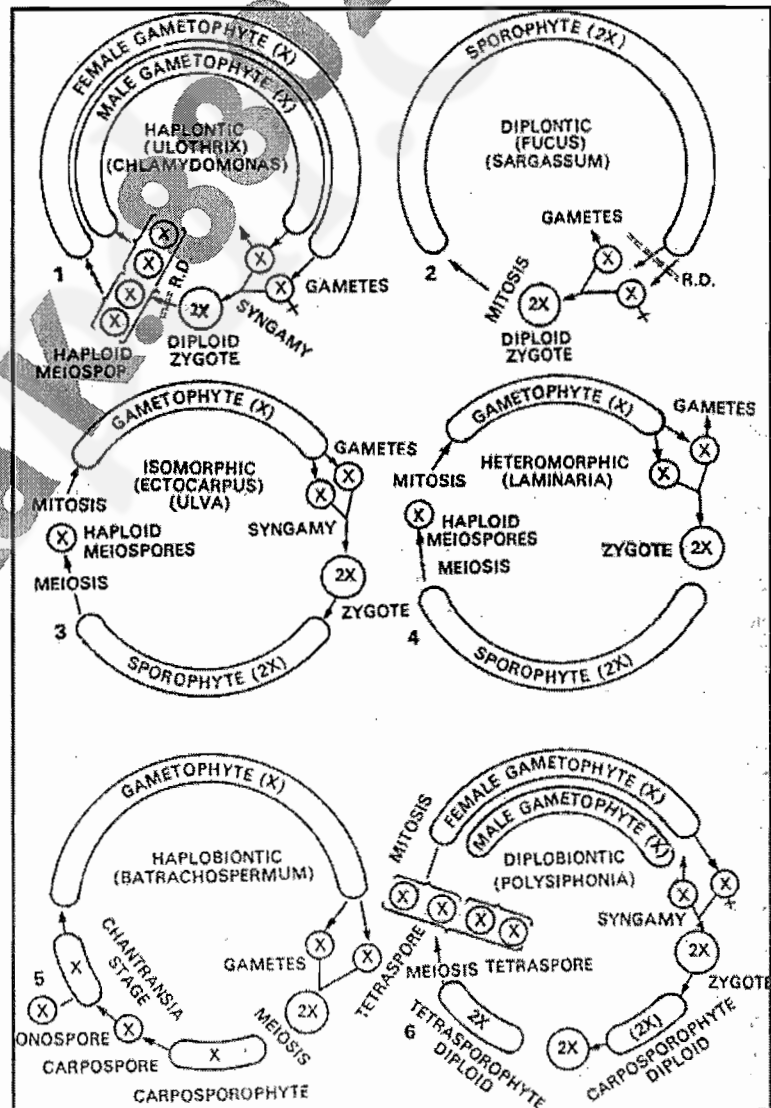
Many life-cycle patterns are found in algae. The representative life-cycle patterns in algae are as follows:

Haplontic type: This is the simplest and most primitive type of life-cycle. The other patterns of life-cycle are believed to have originated from this type. This type is found in all Chlorophyceae except a few. Sometimes this is called *Ulothrix* or *Chlamydomonas* type.

In such cases the somatic phase (plant) is haploid (gametophyte) while the diploid phase (sporophyte) is represented by zygote. During germination the zygote ($2n$) divides meiotically producing haploid (n) zoospores. They develop into individual plants. Here the unicellular (e.g., *Chlamydomonas*) or filamentous (e.g., *Ulothrix*, *Spirogyra*, *Oedogonium*, *Chara* etc.) gametophyte (n) alternates with a one-celled zygote or sporophyte ($2n$). The haploid filamentous plants are known as *haplonts* which reproduce asexually by zoospores or aplanospores.

Diplontic type: This pattern is the reverse of haplontic type. In this case somatic phase (plant) is diploid (sporophyte $2n$). The haploid phase (gametophyte n) is restricted to gametes, produced by meiotic division. After gametic union a diploid zygote is formed, which develops into a diploid (sporophyte $2n$) plant by mitotic division. The well known examples of this pattern are – *Fucus*, *Sargassum*, *Codium*, *Bryopsis* etc.

1. **Isomorphic type:** This is a type of well defined alternation of generations, where there are two exactly similar i.e. morphologically identical somatic phases. Here the one phase is diploid [$2n$], while the other is haploid (gametophyte, n). Among Chlorophyceans this is found in *Ulvaceae*, *Chaetophoraceae* and *Cladophoraceae*. The Brown Algal orders *Ectocarpales*,



Cutleriales & Dictyotales also show this pattern of life-cycle. In such cases, the zygote develops into a diploid multicellular plant (sporophyte) by postponement of meiosis. Prior to zoospore (meiospore) formation there is meiosis. These zoospores develop into haploid plants & complete the life cycle.

2. **Heteromorphic type:** This pattern of life cycle is exactly like that of preceding one (isomorphic type) only with the difference that the **alternating haploid (n) and diploid (2n) somatic phases (plant) are morphologically different**. This pattern is found in Laminariales, Sporochneales, Desmarestiales etc. of Phaeophyceae and *Urospora* of Chlorophyceae.

Haplobiontic type: In this pattern there are **three phases in the life-cycle**. Out of three, two phases are haploid (n) and one diploid (2n). The examples are found among Nemalionales (e.g. *Batrachospermum*) of Rhodophyceae. For example, in *Batrachospermum* haploid gametophytic phase produces gametes which on fusion form a zygote (2n). The latter is the only diploid phase which divides meiotically forming a haploid asexual phase known as carposporophyte. The latter reproduces asexually by haploid carpospores which again develop into gametophytes (haploid plants, n). Thus two morphologically different haploid phases (gametophyte and carposporophyte) alternate with the zygote (2n).

Diplobiontic type: This type of life-cycle is found in almost all Rhodophyceae except Nemalionales. The most common example is *Polysiphonia* of order Ceramiales. Here the **life cycle is triphasic and involves an alternation of two diploid (2n) or sporophytic generations**, [i.e., carposporophyte and tetrasporophyte] **with one haploid (n) or gametophytic generation**. Thus there are two diploid phases and one haploid phase. The gametophyte produces gametes which on fusion form a zygote (2n). Now the zygote divides mitotically forming a carposporophyte.

On germination the diploid carpospores form another diploid plant, the tetrasporophyte. The latter produces tetraspores by meiosis. The haploid tetraspores germinate and give rise to haploid gametophytic plants.

Various uses of algae

Algae in Agriculture

Soil algae, specially the blue-green algae are capable of fixing atmospheric nitrogen and increase the fertility of the soil. They also enhance the crop production. These algae are the chief agents of nitrogen fixation in rice fields. Hence they are known as the bio-fertilizers or algal fertilizers. Species of *Anabaena*, *Nostoc*, *Tolypothrix* and *Aulosira* are important in this field. These are cultivated on mass scale, dried and supplied in packets as seed material to be applied in the rice fields at the time of crop cultivation. The blue-green algae are also useful in the reclamation of barren alkaline soils. By growing the blue-green the alkalinity may be neutralized and fertility may be increased. Likewise such soils are reclaimed and brought under cultivation.

A number of seaweeds are used as fertilizers (as manure). Large brown and red algae are important in this. They are rich in 'Potassium' and poor in 'Nitrogen and 'Phosphorus' than the farm manure. They are applied to the field directly and ploughed. Such fields are used for growing vegetables in countries like France, Ireland and Sri Lanka. In Japan they are used in the rice fields and in China for growing groundnut and sweet potatoes. In India *Turbinaria* is used as a fertilizer for palm trees. The seaweeds are also used as compost. Sometimes the burnt ash is added to the farm lands. The concentrated liquid extracts of some seaweeds are sold as liquid fertilizers and also as insecticides.

Lithothamnion and *Lichmophyllum* which are encrusted with lime, are used in place of lime, after grinding the material. The freshwater alga, *Chara* can also be used similarly.

Algae as Food

Algae serve as a source of food for fishes, aquatic and terrestrial animals and human beings. More than 70 species of marine algae (red and brown seaweeds) have been used for food in oriental countries like China, Japan etc. The red, brown and green algae form a regular portion of human diet and are used prolifically. *Spirogyra* and *Oedogonium* in India and *Ulva* in Europe are important. The mucilage balls or colonies of *Nostoc* are boiled and eaten in Brazil. The raw red and brown algae are chopped and added to other dishes. The young stipes of *Laminaria* and sporophylls of *Alaria* are also eaten in Japan. *Durvillea Ulva* are dried, salted and sold. Large quantities of these are consumed in Chile. *Ulva lactuca* was used in salad and soups in Scotland. *Porphyra* is a tasteful dish in Korea, Japan and China. It is rich in vitamins 'B' and 'C'. In Philippines *Caulerpa* is cultivated as a source of food.

Some algae are rich in proteins, fats and vitamins A,B,C & E. The diatom *Nitzschia* is rich in vitamin A. Vitamin 'B' is common in *Ulva*, *Enteromorpha*, *Laminaria*, *Porphyra* and *Chondrus*. *Ulva*, *Enteromorpha*, *Alaria* etc. also contain vitamin 'C'.

Algae as Fodder

Norway, France, Denmark, New Zealand and U.S.A. use marine algae as fodder for cattle. *Ascophyllum*, *Fucus* and *Laminaria* are processed into suitable cattle feed and given to the cattle, poultry and pigs. This enhances the milk yielding capacity of the cattle. Similarly, butter and fat content of milk increases. The egg laying capacity of the poultry increases and egg-yolks will have increased iodine and carotene content. *Rhodymenia* and *Sargassum* are used as fodder in France and China respectively.

Algae in Medicine

Laminaria species have high iodine content. *Codium* contains a considerable amount of iodine. *Gelidium* and *Grateloupia* also contain iodine. This iodine is used in the preparation of various goiter medicines. Some algae are a source of antibiotics. Chlorellin from *Chlorella* is an antibiotic. But this has not been chemically characterized. The extracts of *Cladophora* and *Lyngbya* possess antiviral and antibacterial properties. The Charophytes possess larvicidal properties. So these plants are useful in destroying mosquito larvae. Because of antibiotic property certain algae were used in phycotherapy, healing of wounds in earlier days.

Agar agar is used in the manufacture of pills and ointments. It also forms a base for many medicines which are used as laxatives. Carrageenin acts as a blood coagulant. Alginic acid controls bleeding. The extracts of *Digenea*, *Codium* and *Durvillea* have vermifuge effect.

Algae in Oil and Gas

The organic compounds derived from the dead plants and animals constituting the plankton accumulate at the bottom and buried in the sediments of oceans. These compounds are decomposed and converted into oil (petroleum) and fuel gas (methane) by the action of methane producing bacteria.

Algae in Sewage Treatment

Chlorella, *Scenedesmus*, and *Euglena* grow very well in domestic wastes and help in converting it into an odourless valuable fertilizer. The algae flourish on the nutrients present in sewage and liberate oxygen during their photosynthesis. The oxygen is used by the microorganisms for the decomposition of organic matter in sewage. Thus the algal-bacterial system helps in the purification and disposal of sewage. Besides this, the algae can be separated from sewage after a certain period of growth, dried and used as a feed for poultry.

Algae in Experimental Work

Algae are used extensively in biological research. The cultures of *Chlorella*, *Scenedesmus*, *Anacystis* and other microalgae have been widely used in the investigations of photosynthesis. The sexual reproduction at the cellular and molecular level has been thoroughly understood through the studies made in *Chlamydomonas* and other Volvocan algae.

Chlorella pyrenoidosa, *Spirulina* and *Synechococcus* can be used as a possible food source in space flights. These algae multiply rapidly, synthesize food by utilizing CO₂ and liberate oxygen.

Algae in Water Supplies

In freshwater ponds, lakes and reservoirs/tanks certain algae (blue-greens, diatoms and euglenoid flagellates) grow in abundance and constitute the water blooms. Such algae impart colour and unpleasant odour to water, thus making it unfit for drinking. Certain algal growths may choke the pipes and interfere with water supplies. Blue-greens like *Microcystis* and *Aphanizomenon* produce toxic substances into the water which are poisonous to fish, cattle etc.

Some biologists have emphasized the role of air-borne algae as causative agents of allergies.

Economically important products from algae

The algae yield a number of products of commercial importance. The major products are 1. Agar agar, 2. Carrageenin, 3. Alginic acid and 4. Diatomite.

Agar Agar

It is a slimy or mucilage substance obtained from the cell walls of certain red algae. It is extracted from the thalli of *Gelidium*, *Gracilaria* and *Gigartina*. Japan was the largest producer of agar agar until 1939. The extract is a gel containing galactose and a sulphate. It melts between 90° and 100°F and solidifies at lower temperatures. Agar agar is used in the preparation of food stuffs such as salads etc. and taken as a dish along with the diet regularly. It is used as an emulsifier in dairy products (ice-cream preparation). It is largely used as a substrate in the culturing of various microorganisms such as bacteria, algae and algae in the laboratories. It is also used in cosmetics, leather and textile industries.

Alginic Acid and Alginates

These are extracted from the marine brown algae such as *Ascophyllum*, *Laminaria*, *Macrocystis* etc. Algin is a carbohydrate present in the middle lamellae and primary walls of the seaweeds. It is a colloidal substance present in the form of a Calcium salt which is soluble. The salts are known as the alginates. These are used as thickening agents in food industry, cosmetics, and in textiles as printing pastes. They are also used in the preparation of plastics and artificial fibres. The alginates have great value as emulsifiers, gelling agents, dental impression powder, paints etc.

Carrageenin

It is a cell wall polysaccharide. It is mucilaginous in nature. It is obtained mainly from the red alga, *Chondrus crispus* (Irish Moss) and in lesser quantity from *Gigartina*. The mucilaginous extract is used in food, textile, pharmaceutical, leather and brewing industries. It is used as a component in tooth pastes, deodorants, cosmetics etc. It is also used to stabilize emulsions.

Iodine

Japan produces iodine regularly from the kelps (brown seaweeds) e.g., *Laminaria*, *Fucus*, *Eisenia* and *Ecklonia*. Similarly bromine can be obtained from the red algae—*Rhodotmela* and *Polysiphonia*.

Minerals

The kelps are also a source of soda and potash. The ash of the kelps will be added to the soil to increase the mineral content of the soil.

Glue

Another important algal industry in Japan is glue manufacturing. For this purpose *Gloeopeltis furcata*, a red alga, is used. This glue is known as 'funori' and is used for sizing paper and cloth. It is also used as an adhesive.

Diatomite

It is a diatomaceous deposit formed due to the deposition of indestructible, siliceous frustules of diatoms over a number of years over the sea floors. This diatomaceous earth has got several commercial uses. It is used as a filter for oils, in sugar industry and for clearing solvents. It is used in the insulation of refrigerators, boilers, hollow tile bricks for the construction of constant temperature rooms, sound proof rooms and in metal polishes. It is also a constituent of some tooth powders, bleaching powders and a reinforcing agent in concrete. It is also used as a base on auto-mo-bile and silver polishes.

Classification by Linda Graham and Wilcox (2004)

The term **Algae** is a *non-taxonomic* expression that applies to predominantly aquatic, simple, chlorophyllous (hence photoautotrophic) organisms which never form an embryo and have their sex organs unprotected by any sterile jacket. Other common characters of the algae include the absence of vascular system (xylem and phloem) or organs like root, stem and leaves. The mode of dispersal is spores (and never the seeds) which are specialized to disseminate in an aquatic medium.

The classification of algae above the order level has changed substantially since 1960 due to:

1. Research using electron microscopes has demonstrated new and important features
2. Molecular studies, especially comparative gene sequencing studies, revealing new phylogenetic affinities

The following classification has been accepted by the *Tree of Life Project*. The ICBN has recently proposed a substitution of the term *Division* by *Phylum* in Algal classification.

Phylum Chlorophyta (green algae)

Chlorophylls *a* and *b*; starch stored inside chloroplast; mitochondria with flattened cristae; flagella, when present, lack tubular hairs (mastigonemes); unmineralized scales on cells or flagella of flagellates and zoospores; conservatively, between 9,000 and 12,000 species.

Class Prasinophyceae

Primarily freshwater, scaly cell wall; includes – *Collinsiella* and *Prasinocladus*

Class Chlorophyceae

Primarily freshwater; includes *Chlamydomonas*, *Chlorella*, and *Oedogonium*.

Class Charophyceae

Includes the macroscopic pondweed *Chara*, filamentous *Spirogyra*, and desmids.

Class Micromonadophyceae

Primarily marine; includes the smallest eukaryotic alga, *Micromonas*.

Class Pleurostrophyceae

Freshwater and marine; includes marine flagellate *Tetraselmis*.

Class Ulvophyceae

Primarily marine; includes sea lettuce *Ulva*.

Phylum Chromophyta

Most with chlorophyll *a*; one or two with chlorophyllide *c*; carotenoids present; storage product β -1, 3-linked polysaccharide outside chloroplast; mitochondria with tubular cristae; biflagellate cells and zoospores usually with tubular hairs on one flagellum; mucous organelles common.

Class Bacillariophyceae (diatoms)

Silica cell walls, or frustules; centric diatoms commonly planktonic and valves radially symmetrical; pennate diatoms found attached to substrate and valves bilaterally symmetrical; primarily in freshwater, marine, and soil environments; at least 12,000 to 15,000 living species; tens of thousands more species described from fossil diatomite deposits; *Cyclotella* and *Thalassiosira* (centrics) and *Navicula* and *Nitzschia* (pennates).

Class Bicosoecophyceae

May be included in the Chrysophyceae or in the protozoan group Zoomastigophora; colourless flagellates found in vase-shaped loricas (wall-like coverings); cell attached to lorica using flagellum as a stalk; lorica attaches to plants, algae, animals, or water surface; in freshwater and marine; fewer than 50 species described; *Bicosoeca*.

Class Chrysophyceae (golden algae)

Many unicellular or colonial flagellates; also capsoid, coccoid, amoeboid, filamentous, parenchymatous, or plasmodial; many produce silica cysts (statospores); predominantly freshwater; approximately 1,200 species; *Chrysamoeba*, *Chrysocapsa*, and *Ochromonas*.

Class Dictyochophyceae

Predominantly marine flagellates, including silicoflagellates, which are common in diatomite deposits; fewer than 25 described species.

Class Phaeophyceae (brown algae or brown seaweeds)

Microscopic forms to large kelp more than 60 metres long; more than 1,500 species, almost entirely marine; *Ectocarpus*, *Macrocystis*, and *Sargassum*.

Class Prymnesiophyceae (Haptophyceae)

Many with haptonema, a hairlike appendage between two flagella; no tubular hairs; many with organic scales; some deposit calcium carbonate on scales to form coccoliths; coccolithophorids may play a role in global warming because they can remove large amounts of carbon from the ocean water; predominantly marine and planktonic species; approximately 300 species; more fossil coccolithophores known; *Chrysochromulina*, *Emiliana*, and *Prymnesium*.

Class Raphidophyceae (Chloromonadophyceae)

Flagellates; mucocysts (mucilage-releasing bodies) commonly found in freshwater forms; sharply divided between freshwater and marine environments; fewer than 50 species; *Heterosigma*, *Vacuolaria*, and *Olisthodiscus*.

Class Synurophyceae

Previously placed in Chrysophyceae; silica-scaled; unicellular or colonial flagellates sometimes alternating with capsoid benthic stage; cells covered with elaborately structured silica scales; approximately 250 species, with approximately 10 new described each year since 1970; *Mallomonas*, *Synura*, and *Tesselaria*.

Class Xanthophyceae (yellow-green algae)

Primarily coccoid, capsoid, or filamentous; mostly freshwater environments; about 600 species; *Bumilleriopsis*, *Tribonema*, and *Vaucheria*.

Phylum Cryptophyta

Unicellular flagellates.

Class Cryptophyceae

Chlorophyll *a*, chlorophyllide *c*₂, and phycobiliproteins; starch stored outside of chloroplast; mitochondria with flattened cristae; tubular hairs on 1 or both flagella; special ejectosomes lie in a furrow or gullet near the flagella; cell covered with periplast, often elaborately decorated sheet or scale covering; nucleomorph may represent reduced nucleus of symbiotic organism; approximately 200 described species; *Chilomonas*, *Cryptomonas*, *Falconomonas*, and *Rhinomonas*.

Phylum Pyrrophyta (Dinoflagellata)

Predominantly unicellular flagellates; approximately half of the species are heterotrophic rather than photosynthetic; photosynthetic forms with chlorophyll *a*, 1 or more chlorophyllide *c* types, and peridinin or fucoxanthin; mitochondria with tubular cristae and flagella without tubular hairs; ejectile trichocysts below surface in many members; many with cellulosic plates that form an armour around cell; some bioluminescent, some containing symbionts; nucleus contains permanently condensed chromosomes; several produce toxins that either kill fish or accumulate in shellfish and cause sickness or death in humans when ingested; more than 1,200 species described, most in the class Dinophyceae; *Alexandrium*, *Dinophysis*, *Peridinium*, and *Polykrikos*.

Phylum Euglenophyta

Primarily unicellular flagellates; both photosynthetic and heterotrophic.

Class Euglenophyceae

Chlorophylls *a* and *b*; paramylon stored outside chloroplasts; mitochondria with paddle-shaped cristae; flagella lack tubular hairs, but some with hairlike scales; unusual pellicle covering of sliding sheets allows organisms to change shape easily; approximately 1,000 described species; *Colacium*, *Euglena*, and *Eutreptiella*.

Phylum Rhodophyta (red algae or red seaweeds)

Predominantly filamentous; mostly photosynthetic but almost one-third parasitic; photosynthetic species with chlorophyll *a*; chlorophyll *d* present in some species; phycobiliproteins (phycocyanin and phycoerythrin) organized into discrete structures (phycobilisomes); starch occurs outside chloroplast; mitochondria with flattened cristae; flagella completely absent; coralline red algae contribute to coral reefs and coral sands; predominantly marine; approximately 4,100 described species; *Bangia*, *Palmaria*, *Polysiphonia*, and *Porphyra*.

Some general features of algae

Occurrence/Habitat

- Universal - in a variety of habitats - such as freshwater, seawater, on snow, on rocks and on or within plant and animal bodies.
- Aquatic forms most common.
- Algae - classified into 3 groups on basis of habitat -
 - a) Aquatic Algae
 - b) Terrestrial Algae
 - c) Algae of unusual habitats

Aquatic Algae

- Fresh water forms - in ponds, pools, lakes, rivers, etc. Eg. - *Cladophora*, *Oedogonium*, *Ulothrix*, *Chara*, *Chlamydomonas*, *Volvox*, etc.
- Marine forms - in saline water of the sea and represented by members of Phaeophyceae (*Ectocarpus*, *Laminaria*, *Fucus*, etc) and Rhodophyceae (eg. *Polysiphonia*).
- Either free-living (eg - *Chlamydomonas*, *Volvox*, *Spirogyra*) or attached to a substratum with a holdfast (eg. - *Oedogonium*, *Ulothrix*).
- Many free-floating forms, with similar forms, form colonies on water surface - called Algal Water Blooms or phytoplanktons.
- Phytobenthos - Algae attached to rocks along edges of lakes or seas.

Terrestrial Algae

- Found in soils, rocks, logs, etc.
- Forms - *Vaucheria*, *Botrydium*, *Fritschella*, and *Euglena* - found on soil surface - called Saphopytes.
- Many blue green algae - *Nostoc*, *Anabaena* - under soil surface - called Cryptophytes.
- Some also found on tree trunks and moist walls. Eg. - *Protococcus*, *Scytonema*.

Algae of Unusual Habitats

- Halophytic Algae - (i) occur in saline water of seas or salt lakes. (ii) Eg - *Chlamydomonas ehrenbergii*, *Dunaliella*.
- Epiphytic Algae - (i) grow on larger algae or on bryophytes and angiosperms. (ii) *Oedogonium* and *Microspora* found attached to larger species of *Cladophora*, *Vaucheria*.
- Epizoid Algae - (i) grow on animals like snails, fishes and tortoise. (ii) *Cladophora crispata* grows on snails; *Characium* found on the legs of *Branchipus*.
- Endozoic Algae - (i) occurs in tissues of animals. (ii) Species of *Zoochlorella* found in *Hydra viridis*.
- Symbiotic Algae - (i) Several members of Chlorophyceae and Cyanophyceae form symbiotic association with fungi, bryophytes, gymnosperms and angiosperms. (ii) Lichens - an association between algae and fungi; their algal components - Chlorophyceae or Cyanophyceae; colonies of *Nostoc* and *Anabaena* - in symbiotic association in the thallus of *Anthoceros* and coralloid roots of *Cycas*.
- Cryophytic Algae - (i) on ice or snow. (ii) Alpine and Arctic mountains become red due to growth of *Hematococcus nivalis*; green snow in Europe due to *Chlamydomonas yellowstonensis*.
- Lithophytic Algae - (i) grow on moist rocks and stones. (ii) Blue green algae *Nostoc*, *Rivularia*.
- Parasitic Algae - (i) grow as parasites on many plants and animals. (ii) *Polysiphonia fastigiata* - a semiparasite on *Ascomyllum nodosum*.
- Thermophytes - (i) many blue-green algae - *Oscillatoria brevis*, *Haplosiphon lignosum* - found in hot-water springs (50-70°C). (ii) Survive in such high temperatures due to absence of well organised nucleus.

Range of Thallus Structure

Vegetative structure of algae shows a wide variety-range in form from unicellular to complex multicellular thalli.

Size range - from 1 micron to several meters.

On basis of thallus organization - algae divided into following 5 groups -

Unicellular forms

- Multicellular forms derived by repeated divisions of unicellular forms.
- In all groups of algae except Charophyceae and Pheophyceae.
- 4 sub -groups -
 - (a) Rhizopodial-lack rigid cell wall; possess cytoplasmic projections which help them in amoeboid movement; eg - Rhizochloris (Xanthophyceae).
 - (b) Flagellated Unicells - Found in all groups of algae except - Cyanophyceae, Pheophyceae and Rhodophyceae.
 - (c) Spiral Filamentous Unicells - eg - Spirulina (Cyanophyceae).
 - (d) Non-motile Unicells - Simplest non-motile forms in Cyanophyceae (eg - Chroococcus), Chlorella, Diatoms.

Colonial forms

- Developed by aggregation of products of cell division within a mucilage mass.
- Sub-groups -
 - (a) Coenobial - (i) Definite shape, size, arrangement of cells colony (ii) Motile colony. Eg. - Pandorina, Volvox, Eudorina. (iii) Non-Motile colony. Eg. - Hydrodictyon.
 - (b) Palmelloid - (i) Number, shape size of cells in colony - indefinite (ii) Eg - Chlamydomonas, Tetraspora, etc.
 - (c) Dendroid - (i) Colony looks like microscopic tree. (ii) Eg - Chrysodendron.
 - (d) Rhizopodial-(i) Cells - united through rhizopodia. (ii) Eg - Chrysidiastrium

Filamentous forms

- Developed by repeated transverse division of cells. Daughter cells remain attached in a definite sequence to form a filament.
- Sub-groups -
 - (a) Unbranched filaments - (i) free floating - eg - Spirogyra; (ii) attached to substratum - eg - Ulothrix, Oedogonium; (iii) form colony - eg - Nostoc, Oscillatoria.
 - (b) Branched - Following sub-types :
 - False branch - eg - Scytonema.
 - Simple filament - eg - Cladophora
 - Heterotrichous-Thallus is very much evolved and differentiated into prostrate and erect systems. Eg - Fritschia, Ectocarpus, Coleochaete, Polysiphonia.
 - Pseudoparenchymatous - Eg - Batrachospermum, Polysiphonia.

Siphonaceous forms -

- Repeated nuclear divisions without cross wall formations leads to siphonaceous forms.
- Thallus - branched, aseptate, coenocytic, tubular filaments.
- Eg - Vaucharia, Botrychium.

I. Parenchymatous forms

- Flat foliose or tubular thalli formed by division of cells of a filament in two or more planes - parenchymatous form.
- Various shapes - flat (eg - Ulva), tubular (eg - Scytosiphon), or complex (eg - Sargassum).
- Growth of thalli is apical (eg - Fucus), intercalary (eg - Laminaria) or trichothallic (eg - Porphyra).

Flagella

Flagella are filamentous small protoplasmic surface appendages found in both prokaryotic and eukaryotic a cell that brings about cellular motility in aqueous environment.

Occurrence in algae

In all groups of algae except Cyanophyceae and Rhodophyceae.

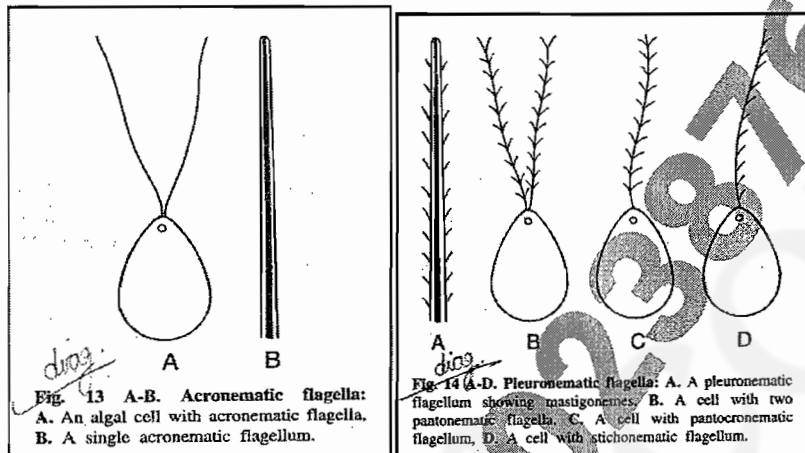
Types

Two types -

- Whiplash or acronematic - have a smooth surface.

- Tinsel or pleuronematic flagella—Surface of these flagella covered with fine filamentous appendages—known as **mastigonemes** or **flimmers**. Further divided into 3 categories on the basis of arrangement of mastigonemes –
 1. Pantonematic – Mastigonemes arranged in two opposite rows or show radial arrangement.
 2. Pantochnematic – Pantonematic flagellum with a terminal fibril.
 3. Stichonematic – Mastigonemes develop only on one side of the flagellum.

A motile cell may have either one or two types of flagella. It is a specific character. If all flagella of a cell are similar then called ISOKONT condition and when dissimilar it is HETEROKONT condition.



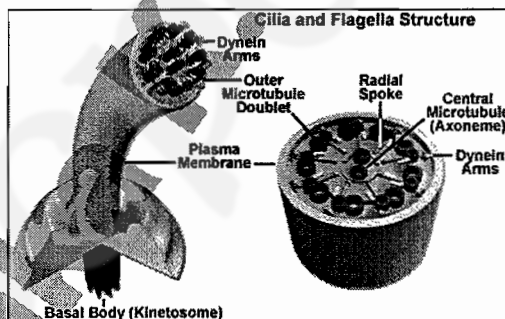
Number

One to four (4) too many.

Motile stages of Chlorophyceae possess two or four anteriorly inserted whiplash flagella of equal length, whereas the members of Phaeophyceae and Xanthophyceae have one whiplash and one tinsel flagellum of unequal length.

Ultrastructure

Transverse section of flagellum reveals – 9 peripheral doublet and 2 central singlet fibrils.



All fibrils are enclosed within a common covering, formed by the extension of plasma membrane, but the two central fibrils have an additional covering of their own. The 9 peripheral fibrils at the proximal end are attached to a hollow basal body (which is separated from the flagellum by a diaphragm). The two central fibrils terminate just short to the diaphragm.

Pigments

A photosynthetic pigment is a pigment that efficiently absorbs light within the 400-700 nm range and is essential for photosynthesis by contributing the absorbed radiant energy for photochemical reactions. There are two types of photosynthetic pigments:

Primary pigment: They include the chlorophylls because only chlorophylls can go through the charge separation reaction in photosynthesis. Chlorophyll is composed of a porphyrin-ring system that is very similar to that of hemoglobin but has a magnesium atom instead of an iron atom (Fig. 1). The algae have four types of chlorophyll, a, b, c (c1 and c2), and d. In some members of Xanthophyceae, chlorophyll e too has been reported. Different chlorophylls have different absorption spectra.

Accessory pigments are light-absorbing compounds, found in photosynthetic organisms that work in conjunction with chlorophyll. Non-chlorophyll accessory pigments include compounds such as carotenoids

or phycobiliproteins, which also absorb light and transfer that light energy to photosystem chlorophyll. Some of these accessory pigments, in particular the carotenoids, also serve to absorb and dissipate excess light energy, or work as antioxidants.

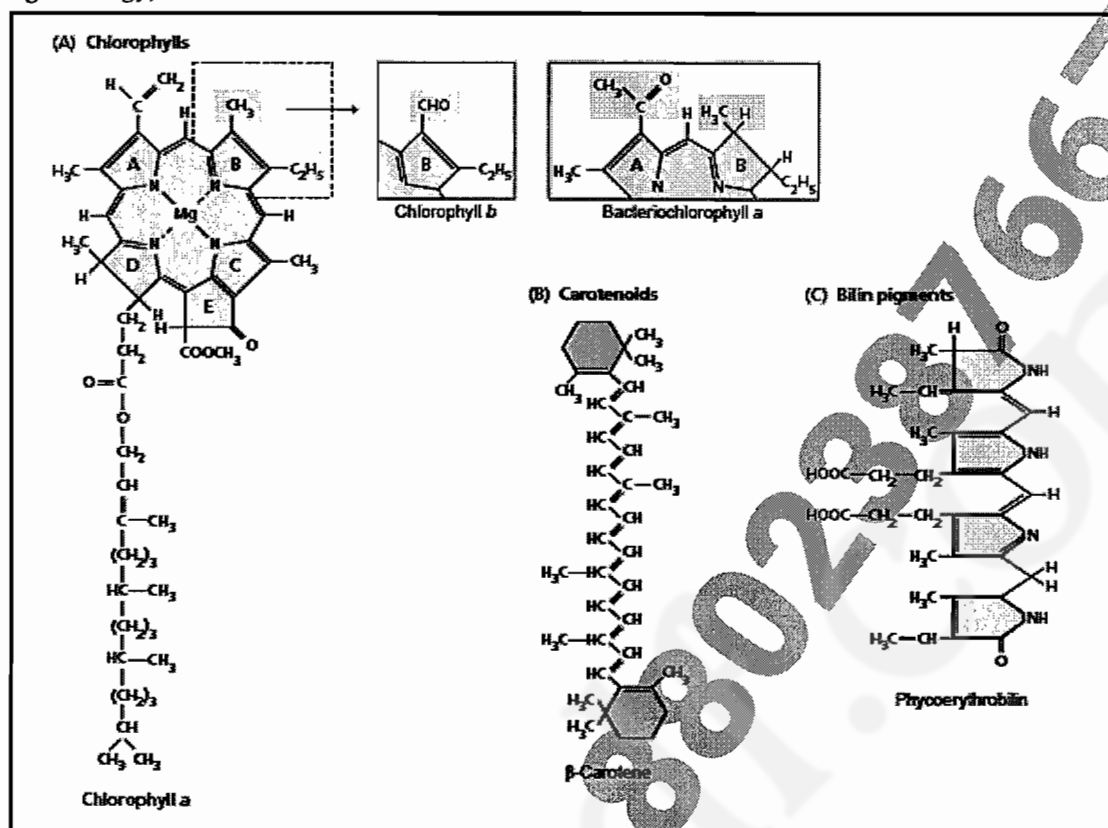


Figure 1: Structure of Some Photosynthetic Pigments

Algal cells have a characteristic color due to the presence of a combination of pigments, specific to each class. In all classes, except Cyanophyceae, these pigments are present within membrane bound organelles known as Plastids. In blue-greens, the pigments are concentrated in the peripheral cytoplasm – called Chromoplasm.

Types of Plastids

1. Leucoplast – Colorless plastids
2. Chromoplast – colored plastids; those containing both chlorophyll 'a' and 'b' – Chloroplasts and those lacking chlorophyll 'b' – Chromatophores.

Different forms of Chromatophores in algae

- Cup-shaped – eg. – *Chlamydomonas*, *Volvox*.
- Discoid – eg. – *Vaucheria*, *Chara*, centric diatoms.
- Girdle-shaped – eg. – *Ulothrix*
- Reticulate – eg. – *Oedogonium*, *Hydrodictyon*, *Cladophora*.
- Spiral – eg. – *Spirogyra*
- Stellate – eg. – *Zygnema*

Types of Pigments in Algal Cells

Chlorophyll

- 5 types – a, b, c, d, e.
- Chlorophyll 'a' in all groups of algae.
- Chlorophyll 'b' – Chlorophyceae, Euglenophyceae.
- Chlorophyll 'c' – largely in algae of marine habitats – Phaeophyceae, Cryptophyceae, Bacillariophyceae and Chrysophyceae.
- Chlorophyll 'd' – some red algae Rhodophyceae.

- Chlorophyll 'e' – in Xanthophyceae such as *Vaucheria hamata*.

Xanthophyll

- More than 20 types known
- Formed by incorporation of molecular oxygen in Carotene molecule.
- Many xanthophylls – common in higher plants – lutein, violaxanthin, neoxanthin – found in members of Chlorophyceae and Phaeophyceae.
- Fucoxanthin – main xanthophyll pigment of Pheophyceae and Diatoms.
- Myxoxanthophyll, Myxoxanthin, and Oscilloxanthin – found only in Cyanophyceae.

Carotenes

- Oxygen free alicyclic compounds of isoprene units.
- 5 types in algae – (a) α -carotene – Chlorophyceae, Cryptophyceae and Rhodophyceae. (b) β -carotene – in all algal groups except Cryptophyceae. (c) γ -carotene – Chlorophyceae. (d) E-carotene – Bacillariophyceae, Cryptophyceae, Pheophyceae, Cyanophyceae. (e) Flavacene – members of Cyanophyceae.

Phycobilins

- Water soluble complexes of protein and bile pigments.
- Present in photosynthetic tissue of plants.
- Are red (phycoerythrin) and blue (phycocyanin) pigments – confined to Rhodophyceae and Cyanophyceae respectively.
- Act as light harvesting pigments in photosynthesis and transfer absorbed light to chlorophyll 'a'.
- Accessory pigments, like carotenoids.

Cyanobacteria (Blue Green Algae)

Introduction to the Cyanobacteria

Cyanobacteria (Greek = blue + bacterium) also known as **Cyanophyta** is a phylum of chlorophyll containing Bacteria. They all have Chlorophyll-*a* and carry out oxygenic photosynthesis. They are also called **blue-green algae**, because they all have chlorophyll, thallus construction and do not form embryo like other algal groups. Their unique blue-green pigmentation is due to the accessory pigments **c- phycocyanin** and **c- phycoerythrin**. They also contain β - carotene. A small group of cyanobacteria, known as *Prochlorophyta* also contain chlorophyll-*b* in addition to chlorophyll-*a*.

The blue green algae are a rather small group with about 175 genera (Morgan, 1998) and 1650 species. In India, the group is represented by 98 genera and 833 species.

Origin and early evolution

The cyanobacteria are one of the earliest living forms on the earth. Fossilized cyanobacteria have been found in rocks more than 3 billion years old. They dominated the earth biodiversity for about 1.5 billion years. Their ability to perform oxygenic photosynthesis converted the early reducing atmosphere of the earth into an oxidizing one. It dramatically changed the life forms on Earth and provoked an explosion of biodiversity. According to endosymbiotic theory of Lynn Margulis (1980), chloroplasts in plants and eukaryotic algae have evolved from cyanobacteria via endosymbiosis.

Cyanobacteria as Algae

The term Algae is a *non-taxonomic* expression that applies to predominantly aquatic, simple, chlorophyllous (hence photoautotrophic) organisms which never form an embryo and have their sex organs unprotected by any sterile jacket. In this sense, the cyanobacteria are definitely algae. However, the cyanobacteria are the only prokaryotic group of organisms regarded as algae. In many fundamental ways the cyanobacteria are different from other algae.

They differ from other algae in the following respects:

1. The blue green algae are photo-autotrophic **prokaryotes** while the rest other algae are eukaryotes. On the basis of their prokaryotic structure, many microbiologists consider blue-green algae as bacteria. Whittaker, in his 5 Kingdom classification system, placed the cyanophytes along with Bacteria in the Kingdom Monera. Studies during the last three decades have firmly established that the cell structure of blue-green algae is entirely different from the members of other algal groups.
2. The pigments in Blue green algal cells are not restricted to definite chloroplasts, but are distributed throughout the peripheral cytoplasm (chromoplasm) or photosynthetic lamella (infoldings of the plasma membrane).
3. These algae are devoid of flagella and their movement is brought about by gliding action. Flagellum is one of the widely occurring cell components in most algal groups.
4. Their cell wall is differentiated into four layers. It is composed of mucoprotein, together with carbohydrates, amino acids and fatty acids. Cellulosic cell wall is absent in the blue green algae.
5. The cell wall is composed of micro fibrils held together in various orientations, such as reticulate (e.g., *Nostoc*) or long intertwining parallel (e.g., *Lyngbya*). While in other algae the cell wall micro fibrils are held together in strictly parallel orientation.
6. A large number of blue green algae are nitrogen fixing, while no other group of algae has N_2 fixing members.
7. There is no incidence of common algal structures like zoospores etc.
8. Sexual reproduction is totally absent from the blue green algae.

However, phycologists regard any organism with chlorophyll, thalloid vegetative body and no embryo formation to be an alga. Therefore, most of the algologists include Cyanophyceae in algae, rather than in bacteria. On the other hand, microbiologists treat these organisms as bacteria. It is now clear that there are considerable differences in the evolutionary history of the cyanobacteria and the rest of the algae as revealed by comparative DNA sequence analysis (Graham & Wilcox, 2000).

Occurrence

The blue-green algae are found in a wide variety of habitats.

- Most of the species are **fresh water**, growing in organically rich permanent waters & form planktons. Some are also found on the bottom of ponds (benthos) & increase fertility of the bottom soil.
- **Marine habitat:** *Dennocarpa* and *Trichodesmium*.
- **Terrestrial habitat:** Species of *Nostoc* and *Oscillatoria*.
- **Endophytes** Some species of *Nostoc* and *Anabaena* grow in roots of *Cycas*, leaves of *Azolla* and thalli of *Anthoceros*.
- **Symbionts:** Species of *Chroococcus*, *Gloeocapsa*, *Nostoc*, *Scytonema* and *Stigonema* are the main algal component (phycobiont) of lichens.

General Characters

Some important distinguishing features of blue-green algae are as follows:

1. They are always unicellular with prokaryotic cell structure, although sometimes they may aggregate to form colonies or chains of cells. In case of a colony, all cells of a colony occur in a common gelatinous matrix.
2. The principal pigments are chlorophyll *a*, *c*-phycocyanin and *c*-phycoerythrin, and they impart characteristic blue-green colour to these algae. A small group of cyanobacteria, known as *Prochlorophyta* also contain chlorophyll-*b* in addition to chlorophyll-*a*. The *prochlorophyta* includes three genera, namely *Prochlorococcus*, *Prochloron*, and *Prochlorothrix*. These genera also contain no blue or red bilin pigment. It was proposed that it is from these cyanobacteria (or their ancient relatives), the chloroplasts in green algae have arisen by endosymbiosis. However, this proposal has not received support from molecular biology. Therefore, the *Prochlorophyta* are considered aberrant cyanophytes (Ralph A. Lewin, 2001).
3. Some members also show a unique ability of Chromatic Adaptation. In this, they can change the secondary pigment constitution of the body according to available light. This ability has also been called *Gaidukov Phenomenon*. It enables these algae to inhabit varying depths in the aquatic habitats.
4. Being prokaryotes, they have no nucleus and membrane enclosed organelle like mitochondria, chloroplasts, ER etc.
5. They lack any kind of membrane bound plastids. Instead, pigments are found embedded within lamellae composed of two membranes joined at the ends. These structures are known as *photosynthetic lamella*.
6. In water columns some cyanobacteria float by forming gas vesicles. These vesicles are not organelles as such. They are not bounded by lipid membranes but by a protein sheath. For this reason, these vesicles are also called *Pseudovacuaules*.
7. Their cell wall is differentiated into four layers. It is composed of mucopeptide, together with carbohydrates, amino acids and fatty acids. Cellulosic cell wall is absent in the blue green algae.
8. The cell wall is composed of micro fibrils held together in various orientations, such as reticulate (e.g., *Nostoc*) or long intertwining parallel (e.g., *Lyngbya*). While in other algae the cell wall micro fibrils are held together in strictly parallel orientation.
9. They lack any type of flagellum. As a result, if they move about it is always by gliding or jerky movements.
10. The reserve food material is a special type of starch (cyanophycean starch) and protein (cyanophycin granules).
11. In some blue-green algae (e.g., *Nostoc*, *Scytonema*) specialised structures, known as heterocysts, are present. Heterocysts are the centre of N_2 -fixation. A large number of blue green algae are nitrogen fixing, though all of them do not contain heterocysts.
12. Reproduction takes place by vegetative and asexual methods. Sexual reproduction is altogether absent. In some genera e.g., *Anabaena*, *Nostoc*, genetic recombination can take place but strictly by parasexual means and not sexual.

Cell Structure

The blue-green algae have prokaryotic cell organisation. The nuclear envelope, which separates DNA from the cytoplasm is totally absent. The cell as such is devoid of organised nuclei, plastids, mitochondria, ER & GA.

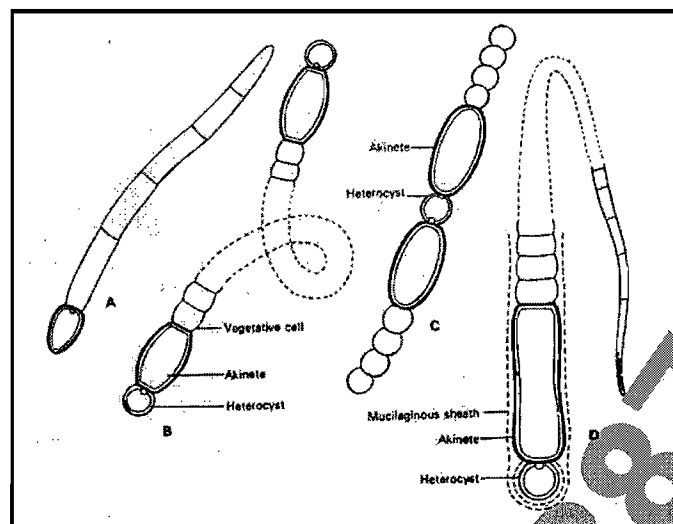


Figure 1: Figure 1: Some forms of Cyanophytes : A- *Anabaenopsis* B-*Cylindrospermum* C- *Anabaena* D- *Gleotrichia*

Sheath and Cell wall The presence of the mucilaginous sheath above the cell wall is a constant feature of blue-green algae. The mucilaginous sheath retains absorbed water which is useful during the periods of desiccation.

A four-layered cell wall is present inside the sheath. The four layers of the wall are designated starting from the inside towards the sheath as L₁, L₂, L₃ and L₄. Each layer is about 10 nm in thickness. In structure and composition the cell wall of blue-green algae is similar to that of Gram-positive bacteria; the cell wall in both is composed of mucopeptide together with carbohydrates, amino acids and fatty acids. The L₂ layer has mainly peptidoglycan.

Plasma Membrane A cell membrane is present inner to the cell wall and this membrane consists of two electron opaque layers separated by a translucent layer. The cytoplasmic membrane invaginates inside the cell, and these invaginations are considered sites of various biochemical functions normally associated with mitochondria, endoplasmic reticulum and Golgi bodies in eukaryotic cells.

Photosynthetic lamellae Lately, EM studies have revealed the presence of a complex lamellar system, which is functionally analogous to the plastids of eukaryotic plant cells. Yet, the lamellae in cyanophycean cells are not separated from the cytoplasmic matrix by a membrane-bounded organelle. They are elongated, flattened sacs consisting of two unit membranes, each about 75 Å thick, separated from each other by a space of 500 Å. They contain Chlorophyll units and phycobilisomes. The phycobilisomes contain accessory photosynthetic pigments, c-phycocyanin and c-phycoerythrin.

Gas Vesicles or Pseudovacuoles In water columns some cyanobacteria float by forming gas vesicles. These vesicles are not organelles as such. They are not bounded by lipid membranes but by a protein sheath. For this reason, these vesicles are also called *Pseudovacuoles*. They are mainly a provision for buoyancy regulation.

Intracytoplasmic inclusions Various kinds of subcellular inclusions in cyanophycean cells include 70S-ribosomes, cyanophycin granules, polyhedral bodies (now called carboxysomes), polyglucoside bodies, polyphosphate bodies, α-granules, β-granules etc.

Nucleoid The central region of a blue-green algal cell appears somewhat less transparent and contains most of the genetic material. This region is regarded as *Nucleoid*. An organised nucleus with a nuclear membrane and nucleolus is absent. DNA is not associated with histone proteins but other basic proteins are present.

Heterocysts

Heterocysts are microanaerobic, N₂-fixing cells that form in a definite

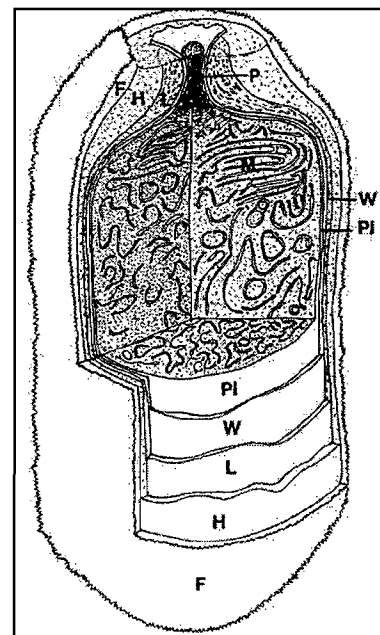


Figure 2: Three-dimensional view of a heterocyst. The envelope has homogeneous (H), fibrous (F), and laminated (L) layers. (M) Membranes; (P) pore channel; (PI) plasmalemma; (W) cell wall. (After Lang and Fay, 1971.):

pattern within N_2 fixing filamentous cyanobacteria such as *Nostoc punctiforme* and *Anabaena sphaerica*, during nitrogen starvation.

Structure Heterocysts are larger than vegetative cells and appear empty in the light microscope (whereas akinetes appear full of storage products). Structural features of heterocysts can be predicted from consideration of their physiology. Since they fix nitrogen from dinitrogen (N_2) in the air using the enzyme nitrogenase and nitrogenase is inactivated by oxygen, so the heterocyst must create a microanaerobic environment. For this, they are surrounded by a thick, laminated cell wall that limits ingress of atmospheric gases, including O_2 . In many cases, they are also covered by several additional layers (Fig 2). The heterocysts are connected to the neighbouring cells by Pore Channels which are partially blocked by Polar Plugs or Pore Plugs.

Formation of Heterocysts Heterocysts are formed at regular intervals from vegetative cells by the dissolution of storage granules, the deposition of a multilayered envelope outside of the cell wall, the breakdown of photosynthetic thylakoids, and the formation of new membranous structures.

The differentiation of heterocysts in *Anabaena* is triggered by nitrogen deprivation (ammonia, nitrate, nitrite) in two steps (Fig. 2). The first step is reversible and the second step is irreversible (Adams, 2000; Kleiner *et al*, 2003).

In the first stage, known as Commitment point 1, the vents are triggered by Nitrogen deficiency. This deficiency leads to a rise in intracellular Ca^{2+} levels. Heterocysts have 10 times more Ca^{2+} than vegetative cells. The rise in Ca^{2+} stimulates the *hetR* gene that forms hetR, a serine-type protease, which induces the vegetative cell to change into a heterocyst. The *hetR* protein is considered the "master switch" in heterocyst development. The production of hetR protein constitutes commitment point 1. It leads to loss of granulation in the cell. This structure is called proheterocyst.

In the second stage, or Commitment Point 2, the proheterocyst is converted into heterocyst. At this stage, the differentiating heterocysts:

1. Produce three additional cell walls, including one of glycolipid that forms a hydrophobic barrier to oxygen. The rate of oxygen diffusion into heterocysts is 100 times lower than of vegetative cells.
2. Produce nitrogenase and other proteins involved in nitrogen fixation. At this stage, *nifD*, *nifK* and *nifH* genes are activated.
3. Degrade photosystem II, which produces oxygen
4. Up regulate glycolytic enzymes, which use up oxygen and provide energy for nitrogenase
5. Produce proteins that scavenge any remaining oxygen inhibit nearby vegetative cells from differentiating into heterocysts. The genes responsible for this are *patA* and *devA*.

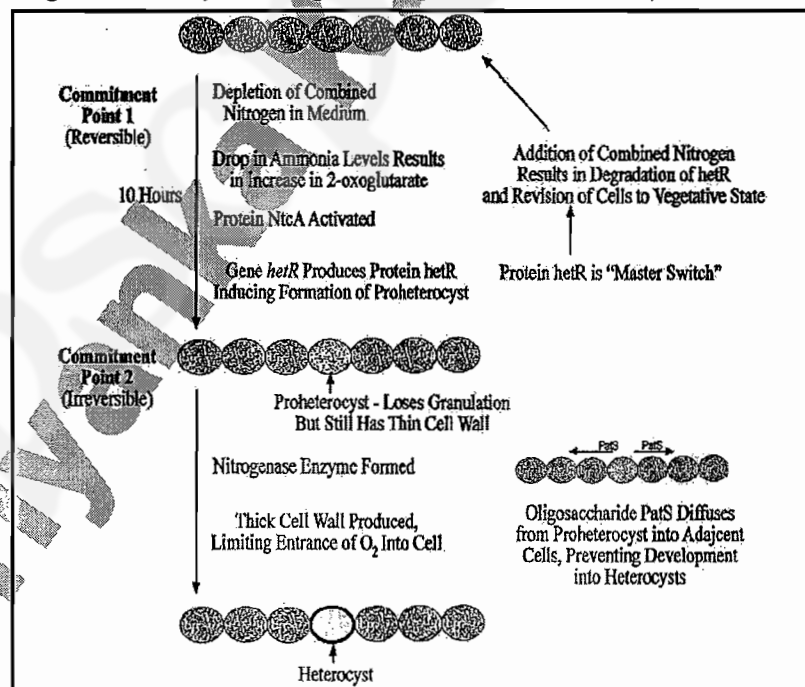


Figure 3: The events leading to heterocyst formation

The frequency of heterocyst production is affected by several factors.

1. The blue and green light inhibit heterocyst development, whereas the white and red light support it.

- The concentration of phosphate salts in the medium stimulates heterocyst development, whereas the absence of Mg and Fe ions in the medium inhibits heterocyst development.
- The presence of combined nitrogen in the medium inhibits heterocyst development. The formation of heterocyst is inversely related to the amount of nitrogen in the medium.
- The cellular C : N ratio plays an important role in inducing and controlling the differentiation of vegetative cells into heterocysts.

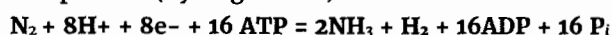
Physiology and Biochemistry Heterocysts are photosynthetically inactive, they do not fix CO_2 , nor do they produce O_2 . They also exhibit a high rate of respiratory O_2 consumption and are sites of N_2 fixation.

Apart from the exceptional cases of germination, heterocysts are unable to divide. Heterocysts have thus *no role in multiplication*, as earlier thought.

Heterocysts are dependent on a supply of substrates from adjacent vegetative cells through cytoplasmic connections (*microplasmodesmata*). These cytoplasmic connections also pass nitrogen fixed in the form of glutamine (Fig. 3) by the heterocysts to vegetative cells. The vegetative cells transfer photosynthetic products to the heterocysts since the heterocysts are incapable of carbon fixation.

In nitrogen fixation, N_2 from the atmosphere is fixed by the enzyme nitrogenase into ammonium using ATP as a source of energy. The process is one of the most metabolically expensive processes in biology, requiring 16 ATP for each molecule of N_2 fixed.

Biological nitrogen fixation can be represented by the following equation, in which two moles of ammonia are produced from one mole of nitrogen gas, at the expense of 16 moles of ATP and a supply of electrons and protons (hydrogen ions):



The nitrogenase enzyme consists of two proteins - an iron protein and a molybdenum-iron protein (Fig. 4).

The reactions occur while N_2 is bound to the nitrogenase enzyme complex. The Fe protein is first reduced by electrons donated by ferredoxin. Then the reduced Fe protein binds ATP and reduces the molybdenum-iron protein, which donates electrons to N_2 , producing $\text{HN}=\text{NH}$. In two further cycles of this process (each requiring electrons donated by ferredoxin) $\text{HN}=\text{NH}$ is reduced to $\text{H}_2\text{N}-\text{NH}_2$, and this in turn is reduced to 2NH_3 .

Importance of heterocysts Biological nitrogen fixation is the most important source of nitrogen in the trophic chains. The amount of biologically fixed nitrogen produced is in excess of 2×10^{13} g per year. It is estimated that about 25% of biotic nitrogen fixation is based on heterocysts.

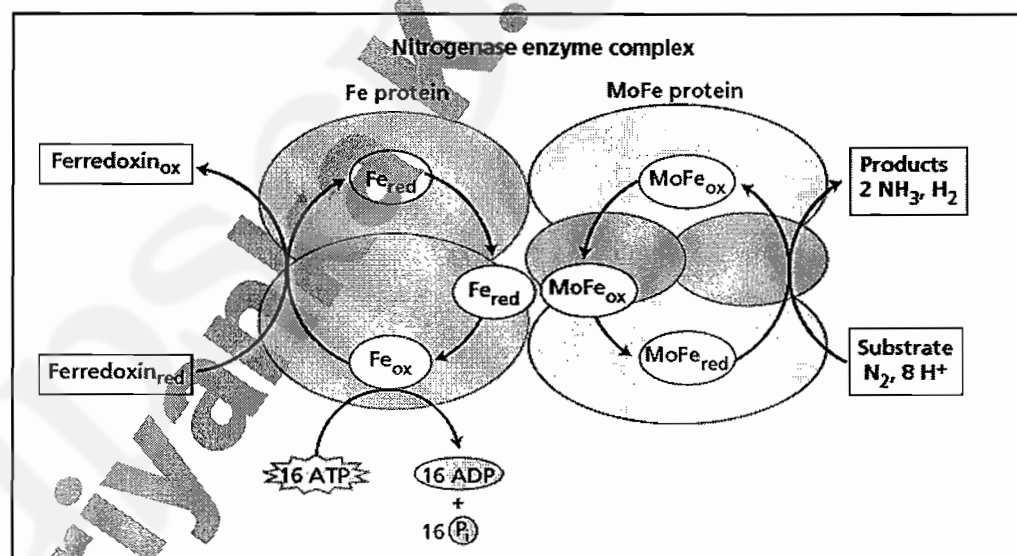


Figure 4: The reaction catalyzed by nitrogenase. Ferredoxin reduces the Fe protein. The Fe protein reduces the MoFe protein, and the MoFe protein reduces the N_2 .

Reproduction in Cyanobacteria

Like bacteria, the cyanobacteria also reproduce asexually only. The most common mode of reproduction is transverse binary fission.

In addition, there are certain specialized structures such as:

1. **Akinetes:** Most filamentous cyanobacteria develop perennating (dormant structures) in adverse condition. These structures are larger than the vegetative cells, are equipped with thick walls, and are called **akinetes**. When favourable conditions return, they germinate and produce new filaments.
2. **Hormogonia:** All filamentous Cyanobacteria reproduce by fragmentation of their filaments (trichomes) at more or less regular intervals to form short pieces each consisting of 5-15 cells. These short pieces of filaments are called **hormogonia**. They show gliding motility and develop into new full-fledged filaments.
3. **Hormocysts:** They are multicellular structures having a thick and massive sheath. They may be intercalary or terminal in position and may germinate from either end or both the ends to give rise to the new filaments.
4. **Spores:** Nonfilamentous cyanobacteria generally produce spores such as endospores, exospores and nanocysts which contribute by germinating and giving rise to new vegetative cells when the unfavourable condition is over.

Phylogeny and Affinities of Cyanophyceae

In the 1960s, when fundamental differences in cellular organisation between prokaryotes and eukaryotes were fully elucidated, it became evident that blue green algae were more closely related to the bacteria than to the eukaryotic algae. However, due to the nature of their photosynthetic pigments and capacity to perform oxygenic photosynthesis they also kept being treated as algae.

The cyanobacteria show several fundamental differences from rest of the algae, which all are eukaryotic. Yet, similarity of blue-green algae with red algae in certain characters, such as the presence of similar phycobilins and complete absence of flagellated reproductive cells suggest some affinity in these two groups. However, some other important characters of blue greens, such as prokaryotic cell structure, and the presence of heterocysts, hormogonia and akinetes are not found in red algae. This indicates that there does not appear any close relationship between these two groups.

Similarities with red algae

1. Flagellated motile cells are absent in both groups.
2. The blue (c-phycoyanin) and red (c-phycoerythrin) pigments present in Cyanophyceae are chemically similar to those found in Rhodophyceae (r-phycoyanin and r-phycoerythrin).
3. Pit connections are present in the family Stigonemataceae of the class Cyanophyceae, and similar structures are also found in Rhodophyceae.
4. The mechanism of fatty acid synthesis is similar in both groups.

Dissimilarities with red algae

1. The blue-green algae show prokaryotic cell structure, whereas it is typical eukaryotic in the red algae.
2. Cells of the blue-greens are covered with a mucilaginous sheath, which is absent in the red algae.
3. Presence of hormogonia and heterocysts is a characteristic of the most blue-greens. But these structures are not found in the red algae.
4. Sexual reproduction is absent in the blue-green algae, whereas the red algae show an advanced type of sexual reproduction.

Affinities with bacteria

1. Both, bacteria and blue-greens show typical prokaryotic structure.
2. Like bacteria, the blue-green algae have an incipient nucleus (nuclear material without nuclear membrane), and lack membrane bound plastids.
3. The mucilaginous sheath surrounding blue-green cells and the capsule present in many bacteria have a similar structure, as both are made up of extremely fine fibrils.
4. Some bacteria (e.g., *Thiothrix*) are structurally similar to hormogonia, found in the blue-green algae.
5. *Beggiatoa*, a sulphur bacterium, resembles with *Oscillatoria* in shape and movement.
6. Like bacteria, the blue - green algae are also sensitive to antibiotics.
7. Many metabolic processes (e.g., sulphur and nitrogen metabolism) are similar in both groups.
8. Recombination of genetic material, similar to bacteria, has been reported in some blue-greens (e.g., *Anacystis nidulans*).

Reproduction in Chlamydomonas

Reproduction in Chlamydomonas

Chlamydomonas is a genus of unicellular green algae (Chlorophyta). These algae are found all over the world, in soil, fresh water, oceans, and even in snow on mountaintops. Algae in this genus have a cell wall, a chloroplast, an "eye" that perceives light, and two anterior flagella with which they can swim using a breast-stroke type motion. More than 500 different species of *Chlamydomonas* have been described of which *C. reinhardtii* are very well studied lab organism (See the Box 1).

Chlamydomonas sp. reproduces by:

1. Asexual and
2. Sexual methods.

The sexual mode of reproduction in this genus has held the interest of biologists, primarily because this alga displays a possible transition from the asexual mode to the sexual mode of reproduction.

Asexual reproduction in *Chlamydomonas* occurs primarily by Zoospore formation. However depending on the growth stage and environmental conditions some other types of asexual spores, such as Aplanospores, Hypnospores and Palmella stage are also found.

a) **Zoospores** (Fig. 1) are formed under favourable conditions when nutrient supply is adequate & temperature is between 20° – 30° C. the process of zoospore formation proceeds as follows:

- The cell withdraws its flagella and becomes non-motile. Soon, the protoplast withdraws from the cell wall.
- It now divides longitudinally into two daughter protoplasts.
- This is followed by the successive longitudinal divisions at right angles to each other, forming 4, 8, 16 or more uninucleate protoplasts within the parent cell wall.
- Each of these protoplasts secretes a thin wall around itself, develops flagella and contractile vacuoles. These flagellate daughter cells are similar to the parent cell in structure and shape, but of smaller size.
- These daughter cells are zoospores, which are released on gelatinization or by rupture of the parent wall. For release from the parent cell, the zoospores secrete an enzyme called **Autolysins**.
- The zoospore after a brief swimming period increases in size and matures into a new cell, which becomes capable of producing new zoospores after 24 hours. Thus a single cell may produce as many as 200,000 daughter cells within a week.

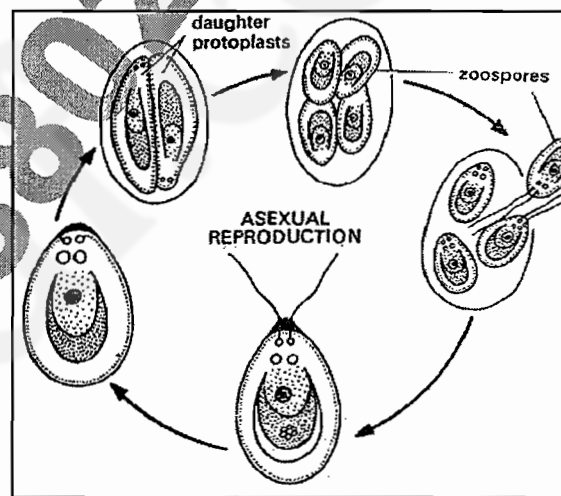


Figure 1: Zoospore formation in *Chlamydomonas*

b) **Aplanospores**: These spores are formed during moderately harsh conditions such as a mild drought. Here the protoplasts divide into 2-16 daughter protoplasts but during maturation do not develop flagella. These non-motile cells with moderately thickened wall are aplanospores.

c) **Hypnospores**: In some species (e.g., *C. nivalis*), aplanospores secrete a thick wall under conditions of severe drought. These very thick walled non-motile spores are called hypnospores. They germinate under favourable conditions and form new cells directly or their protoplasts divide to form zoospores. In certain species, such as *C. nivalis* the wall of the hypnospore is provided with haematochrome, a red pigment, imparting red colour to the hypnospore. This is responsible for the phenomenon of red snow.

d) **Palmella stage**: Under adverse conditions of low humidity and a temperature between 35° – 40° C, the protoplast of the parent cell divides to form 4-8 daughter cells. These cells do not develop flagella and are non-motile and remain within the matrix formed by the gelatinization of the parent wall. This temporary colony of hundreds or thousands of cells, in a common gelatinous matrix, is called palmella stage, because it morphologically resembles an algal genus *Palmella*. The palmella stage is a non-motile and temporary reproductive phase. With the recurrence of favourable conditions, these cells form

flagella and become motile. They come out of the gelatinous matrix and develop into large vegetative cells.

Sexual reproduction: Sexual reproduction in *Chlamydomonas* has been seen to take place in nutrient deficient conditions, which led many workers to propose the hunger theory of sex. In method, it varies from isogamous to primitive oogamous.

- a) **Isogamy:** Most species of *Chlamydomonas* are isogamous, characterised by structural similarities between the fusing gametes. The sexual process starts with the division of the cell protoplast into 8, 16, 32 or 64 biflagellate gametes. The gametes are usually without wall.

Isogamy can be Homothallic or Heterothallic. In homothallic species (e.g., *C. debaryanum*, *C. longistigma*) the gametes produced in the same parent cell fuse to form the zygote, whereas in heterothallic species (e.g., *C. moewusii*, *C. reinhardtii*) gametes from the cells of two different strains fuse (Fig. 2).

In recent years, it has been known that the flagella of gametes are covered by **agglutins**, the chemical substances involved in the recognition of gametes of the opposite strains. These substances are not present in the flagella of the vegetative cells. In heterothallic species when gametes of 'plus' strain come in contact with those of 'minus' strain, the flagella of two gametes of opposite strains adhere because of agglutins. Initially, the gametes clump in groups of up to 50 with their flagella towards the center. In each clump the number of plus and minus gametes is variable. Eventually, gametes of opposite strains fuse in pairs at their anterior ends. The flagella become free and the paired gametes swim away from the clump.

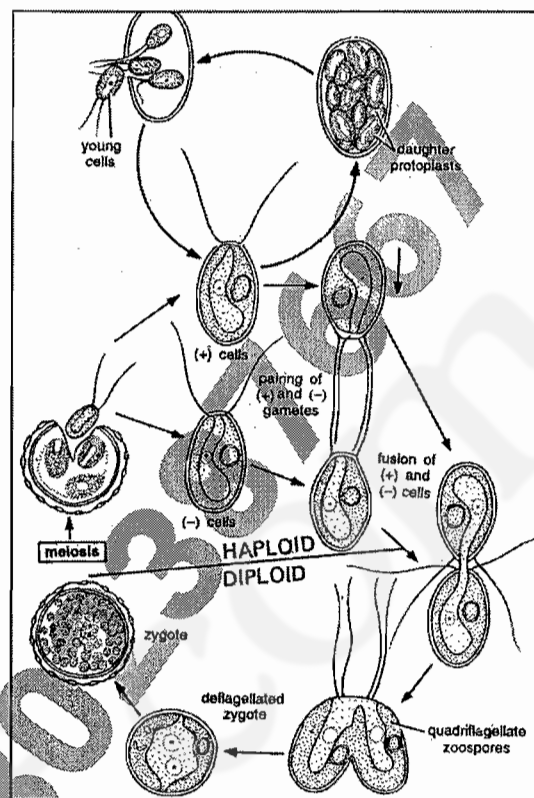


Figure 2: Isogamous heterothallic sexual fusion in *C. reinhardtii*

- b) **Anisogamy:** (Fig. 3) here the fusing gametes are of different sizes but both of them are motile and behave by and large similarly. *C. braunii* and *C. suboogama* are the typical examples of anisogamy. In the female gametangium only two or four macrogametes are formed. In the male gametangium 8-16 microgametes are formed. The micro gametes are more active than the macro gametes and the latter come to rest sooner than microgametes. The active male gamete comes close to the female gamete and both fuse at their anterior ends to form a zygote.
- c) **Oogamy:** (Fig. 3) In *C. coccifera* and *C. ooganum*, the sexual reproduction is of oogamous type. Here the female cell withdraws its flagella and directly functions as a non-motile macrogamete (it is equivalent to egg or ovum). The protoplast of the male cell divides repeatedly to form 16 segments. Each segment develops into a biflagellate micro or male gamete. The flagellated male gamete is active and comes close to non-motile female gamete. The two fuse at their anterior ends to form a diploid zygote.

Zygote: The diploid zygote is initially quadriflagellate. It swims for a while before it settles down and secretes a zygote wall around itself, which is two layered & extensively ornamented on maturity. As the zygote matures, it accumulates large amounts of oils and starch and turns reddish in colour.

Light and carbon dioxide are essential for the development of the zygote. In some species the zygote enlarges in size enormously before germination.

The zygote germinates inside water in dark conditions. Before germination its diploid nucleus undergoes meiosis and consequently four haploid nuclei are formed. In heterothallic species 'plus' and 'minus' strains become distinct at this stage. Each haploid nucleus with some protoplast forms a biflagellate zoospore. Thus, four zoospores are formed in each zygote, but in *C. reinhardtii* eight zoospores are formed in a zygote. The inner wall layer of the zygote is dissolved and the zoospores are liberated when the outer sculptured outer wall splits open. Each zoospore develops into a new cell, thus completing the life cycle.

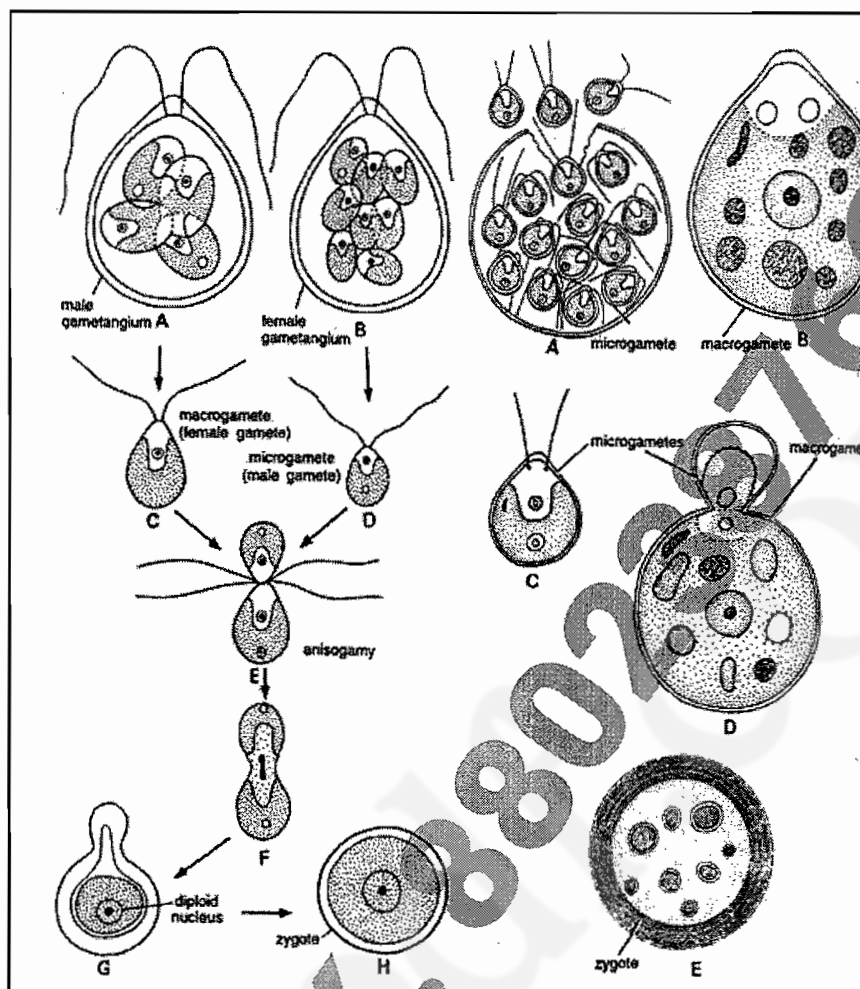


Figure 3: Anisogamous and Isogamous reproduction in *Chlamydomonas*

In *Chlamydomonas* sexuality is controlled by environmental and genetic factors. The amount of ammonium nitrogen in the growing medium is the most important. Its deficiency in the medium inhibits sexuality in *Chlamydomonas* and the gametes behave simply as vegetative cells. Besides this, the intensity of light and temperature, and the presence of calcium in the medium also affects mating of gametes. In high light intensity larger number of zygotes is formed than in low light intensity.

The fusion occurs between the gametes of opposite strains (+ & -) in heterothallic species. It has been observed that non-flagellate mutant forms of *Chlamydomonas* are completely asexual. This suggests that flagella are important in mating processes. The presence of agglutin coating on the surface of flagella probably is essential for sexual processes.

Origin and Differentiation of Sex in *Chlamydomonas*

The various reproductive processes exhibited by different species of *Chlamydomonas* throw light on the evolution of sex in algae. When environmental conditions are favourable, *Chlamydomonas* produces motile, biflagellate zoospores, each of which is capable to develop into a new individual. In this process, no fusion of cells occurs and hence it is a method of asexual multiplication. However, at the close of the growing season when environmental conditions become unfavourable *Chlamydomonas* resorts to sexual reproduction. It involves fusion of two sex cells resulting in the formation of a thick-walled resting structure, the zygote. The resting period of the zygote generally coincides with the duration of the unfavourable period for growth. The cells which fuse to form zygote are called gametes. The origin of gametes is as such the origin of sex.

In isogamous species of *Chlamydomonas* like *C. debaryanum*, *C. moewusii* and *C. longistigma*, isogametes are formed in a similar manner as zoospores. Both the zoospores and gametes are remarkably similar in their form, structure, development and mode of liberation. They differ only in their size and subsequent behaviour. The gametes are invariably smaller in size and are incapable of growing into a new individual alone. The marked resemblances between the gametes and zoospores suggest that isogametes have

originated by the subdivision zoospores into still smaller swarmers in response to unfavourable environmental conditions. These smaller swarmers (reduced zoospores) have become too small and weak to grow into new plants by themselves due to insufficiency of stored food and perhaps some other factors. Most of these perish in their wanderings, but a few meet by chance and fuse in pairs. This pairing increases their vitality and vigour to give rise to a new individual. This clearly illustrates that gametes are just reduced zoospores which are incapable to develop into new individuals. These reduced zoospores are transformed into gametes and their union brings about sexual reproduction: The sex in *Chlamydomonas* is thus originated by accidental fusion of undersized zoospores in response to unfavourable environmental conditions. Since the process proved to be advantageous, it was maintained. During further advancement gametes became of dissimilar size as in *C. braunii*. This was a step towards anisogamy.

The next step in the evolution of sex is demonstrated by *C. coccifera*. Here, a vegetative cell instead of dividing enlarges and loses its flagella and motility. This cell acts as an ovum (egg cell), and fertilized by an active male gamete (antherozoid) formed like zoospores. Of these two gametes, one (the ovum) becomes sluggish and stores food material to support the new plant until it is established on its own, and the other (the male gamete or antherozoid) attains motility to reach the immotile ovum for fertilization. Thus, *C. coccifera* provides an example of the most advanced level of sexuality that can be traced in *Chlamydomonas*.

Volvox

Introduction

Volvox is a fresh water inhabiting, coenobial green alga belonging to the family Volvocaceae under the order Volvocales.

Salient features of Volvocales

1. There are more than 60 genera and 500 species of Volvocales. Almost all of them are fresh-water in habit and frequently they develop luxuriantly in waters rich in soluble nitrogenous compounds.
2. The Volvocales are the only order in Chlorophyta in which the vegetative cells are flagellated and actively motile. The remaining orders of Chlorophyta have non-motile vegetative phase.
3. Some genera are unicellular like *Chlamydomonas*; while other genera are multicellular.
4. The multicellular genera like *Volvox*, *Eudorina*, *Pandorina* are with the number of cells in a colony a multiple of two and with the cells arranged in a definite manner.
5. Most genera have more or less ovoid cells, but some have cells that are compressed or with an irregular outline.
6. Some genera have naked protoplasts, but most of them have a definite cell wall with a cellulose layer next to the protoplast. Frequently there is a layer of pectic material external to the cellulose, and in colonial genera the pectic layers around the individual cells may be completely fused with one another to form a homogeneous colonial matrix.
7. The general organization of the protoplast throughout the order is more or less like that of *Chlamydomonas*. However, there are a few genera, including *Polytoma*, which lack photosynthetic pigments and where the nutrition is saprophytic.
8. Asexual reproduction in unicellular genera is by division into a definite number of cells, and in colonial genera is by all or by certain cells of a colony dividing and redividing to form a daughter colony.
9. Sexual reproduction may be isogamous, anisogamous, or oogamous.

The Volvocales are generally divided into five or six families.

Salient features of the family Volvocaceae

1. The family includes some 10 genera and 30 species, all of them freshwater.
2. The Volvocaceae include all motile colonial genera in which the cells lie in a disk or a hollow sphere and not in superimposed tiers. The number of cells in a colony is definite, a multiple of two, and there is no increase in number of cells after the juvenile phases of development.
3. In asexual reproduction all or certain specific cells divide simultaneously to form daughter colonies.
4. Sexual reproduction is isogamous, anisogamous, or oogamous. All or only certain cells of a colony may be gametogenic.
5. Vegetative cells are always biflagellate, and almost always with a structure like that of *Chlamydomonas*. However, the flagellar insertion in the colonial genera is parallel, whereas it is V type in unicellular genera. Parallel flagellar insertion is considered to be an adaptation for the colonial mode of existence as it minimizes the chances of flagellar entanglement.
6. Cells of all genera have a gelatinous sheath, and abutting sheaths may be distinct from one another or confluent to form a homogeneous colonial matrix. Certain species of one genus (*Volvox*) have conspicuous cytoplasmic strands connecting the cells one to another.
7. All colonies are coenobia (colonies with a definite number of cells arranged in a specific manner). Coenobia of most genera exhibit a definite polarity, when swimming through the water, the anterior pole of the ellipsoid or globose colony always being directed forward.
8. There may also be a definite morphological anterior-posterior differentiation, either in size of eyespots at opposite poles of the coenobium or in outline of the colonial envelope. In certain advanced genera all cells toward the anterior end are vegetative and reproductive cells lie toward the posterior pole (as in *Volvox*).
9. Daughter coenobia are always formed by repeated division of a single cell and according to a definite sequence. All cells of a colony may be capable of forming daughter coenobia, or the capacity to form them may be restricted to specific cells (gonidia) much larger than vegetative cells. Successive divisions in formation of a daughter coenobium are always longitudinal and all cells of

each cell generation divide simultaneously. The four cells of the second cell generation are quadrately arranged; the eight of the third cell generation are cruciately arranged and with a tendency to form a curved plate, the **plakea**. In almost all genera, the plakea becomes a hollow sphere with a small pore, the **phialopore**, at one pole.

Volvox: A brief life history

Ecology

Volvox, a genus with about 20 species, is found in both temporary and permanent fresh-water pools. Sometimes it is present in sufficient abundance to color the water green. *Volvox* usually appears in the spring, increases in abundance, and then abruptly disappears early in the summer. During the remainder of the year it is in a *resting zygote* condition.

Somatic structure

The colonies are called coenobia which spherical to ovoid and with mostly *Chlamydomonas* like cells in a single layer just within the periphery of the gelatinous colonial matrix (Fig. 1). According to the species, the number of cells may be as low as approximately 512 or as high as approximately 65,536 in *V. globator*. Each cell is surrounded by a gelatinous sheath of its own, and the sheaths are usually confluent with one another. In most cases, the sheaths are angular by mutual compression and usually hexagonal. There is gelatinous material of a more watery consistency internal to the gelatinous sheaths of the cells. Most species have ovoid cells. Some species have the cells joined one to another by conspicuous or delicate cytoplasmic strands, a connection which becomes established early in development of colonies.

Most of the cells in a colony are vegetative in nature and are incapable of giving rise to new colonies. Each vegetative cell is biflagellate and with the two contractile vacuoles near the base of the flagella, or with two to five contractile vacuoles irregularly distributed in the anterior end of the cell. The flagellar insertion is parallel. Parallel flagellar insertion is considered to be an adaptation for the colonial mode of existence as it minimizes the chances of flagellar entanglement.

There is either a cup-shaped or laminate chloroplast toward the posterior pole of a cell, and it usually contains but one pyrenoid. Each vegetative cell has a single anteriorly located eyespot, those of cells toward the anterior end of a colony being somewhat larger than those in cells at the posterior end.

Young colonies have all cells alike in size. As a colony grows adult, it has two cell types: about 98% small, biflagellate somatic cells that provide the organism with motility, and about 2% large non-ciliated asexual reproductive cells called *gonidia*.

Asexual reproduction

In asexual reproduction (Fig. 2), there is a differentiation of 2 to about 50 asexual reproductive cells (*gonidia*) in the posterior half of a colony. A gonidium divides longitudinally and all succeeding divisions are longitudinal and simultaneous. The 8-celled stage is the usual cruciate plakea, and the 16 celled stages is a hollow sphere with a phialopore at the anterior pole. Simultaneous division continues for several cell generations.

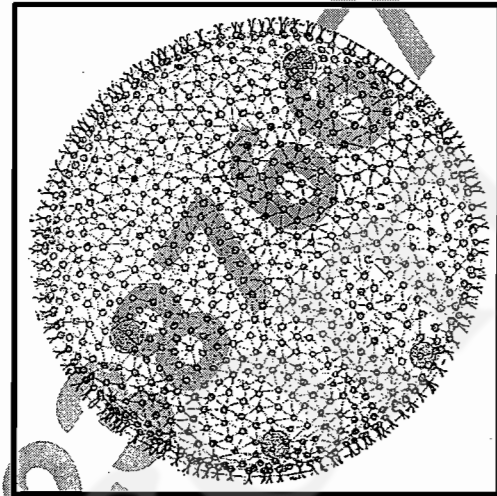


Figure 1: *Volvox* somatic structure

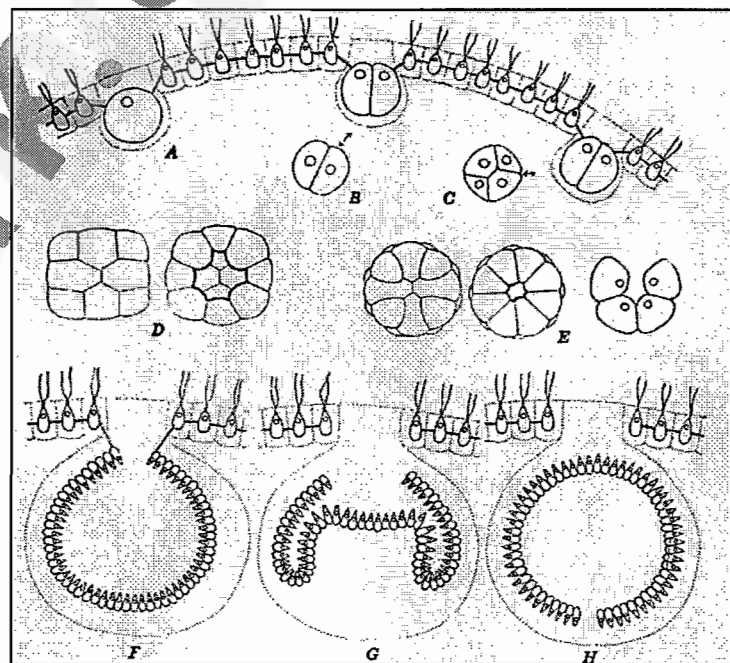


Figure 2: Various stages in *Volvox* asexual reproduction: A. Gonidial differentiation B. The first division C. The second division D. The 8-celled Plakea Stage E. Further division F. Phialopore differentiation at 32 cell stage G. beginning of Inversion H. Completion of Inversion

As shown by Choi *et al* in 1996 and Kirk in 1999, the separation of gonidial and somatic cells is a genetically defined event that occurs after the fifth cell division. The first five divisions are symmetrical, generating a 32-celled stage containing cells of the same size and shape. At the sixth division, each of the posterior 16 cells divides symmetrically to produce 32 similarly shaped cells. However, the anterior end of the structure divides asymmetrically to produce a large "gonidial initial" and a smaller "somatic initial." Each of the gonidial initials divides asymmetrically two to three times (setting off another somatic initial cell at each division). The somatic initials (plus the cells in the posterior end of the embryo) continue to proliferate until a total number of divisions have been completed. As a result, the gonidial initials with about 30 times the volume of the somatic cells are created.

In the separation of gonidial and somatic cells, the following genes have been identified.

1. **Multiple gonidia** (*mul*) mutations in which cause a shift in the time and/or placement of asymmetric divisions, thereby generating more than the normal number of gonidia.
2. **Gonidialess** (*gls*) mutations in which cause no asymmetric divisions and no gonidial initial cells are formed.
3. **Somatic regulator** (*regA*) mutations in which cause somatic cells redifferentiate as gonidia
4. **Late gonidia** (*lag*) mutations in which cause the large cells produced by asymmetric divisions first differentiate as big somatic cells and only later become gonidia.

When cell division ceases, the young colony turns itself inside out by invaginating (**inverting**) through the phialopore. Flagella are developed shortly after inversion, and the daughter colony then revolves slowly within the enlarged gelatinous sac originally containing the gonidium. A daughter colony eventually escapes by moving through a pore-like opening at the free face of the sac. Quite often, as shown by Kirk in 1995, the process of inversion and escape from the phialopore occur simultaneously.

Sexual Process

Sexual reproduction is oogamous (Fig. 3), and according to the species the colonies are homothallic or heterothallic. It is stimulated by somewhat elevated water temperature.

After heat induction of the sexual process, certain coenobia are differentiated to become female coenobia. In such coenobia, a small percentage of cells in a colony develop into eggs. These cells enlarge somewhat, lose their flagella, and resemble young gonidia. These cells also produce a Glycoproteinaceous pheromonal substance, which stimulates the neighbouring undifferentiated coenobia to start producing antherozoids. Later on during the sexual process, the same pheromone also acts as a chemoattractant for the antherozoids.

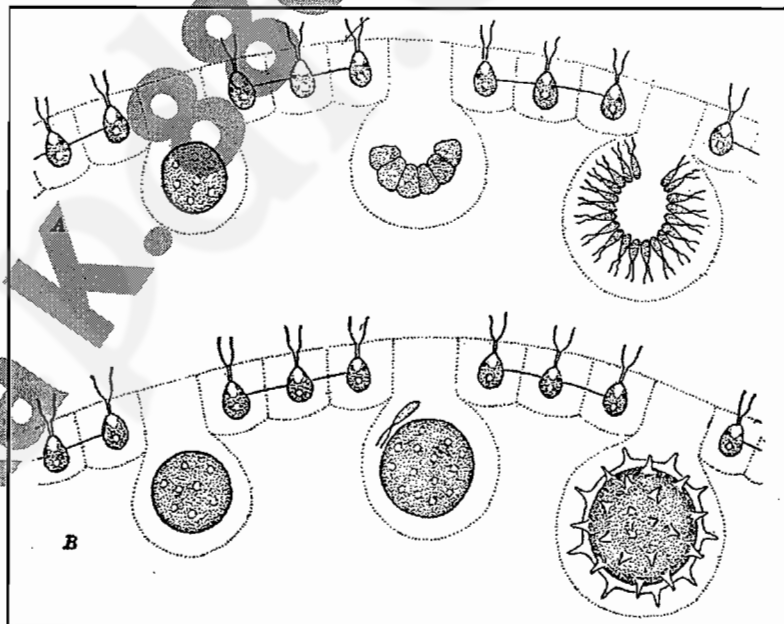


Figure 3: The sexual process in *Volvox*. A. Male development B. Female development

The antherozoids are developed from enlarged cells resembling gonidia. Usually there are relatively few of these cells, but in certain species a majority or all cells of a colony may produce antherozoids. According to the species the cell divides to form 16, 32, 64, 128, 256, or 512 fusiform biflagellate antherozoids. Cell division is in a plakeal sequence and with an inversion to form a bowl-shaped or a globose mass of antherozoids. The colony-like mass of antherozoids is liberated as a unit, swims about as a unit, and does not break up into individual antherozoids until it approaches the vicinity of an egg.

When fertilization takes place the individual antherozoids swim slowly through the gelatinous sheath around an egg and probably enter it from the side. There may also be a development of unfertilized eggs into parthenospores.

After fertilization, the zygote forms a smooth or stellate thick wall and develops sufficient *hematochrome* to color the protoplast an orange red. Zygotes do not germinate until a considerable time after they are liberated from the colony by disintegration of the gelatinous matrix of the colony. Prior to germination there is a meiotic division of the zygote nucleus.

When a zygote germinates there is a splitting of the outer wall layer (exospore) and an extrusion of the inner wall layer (endospore) as a vesicle which surrounds the protoplast. The protoplast may become a biflagellate zoospore, but it rarely escapes from the vesicle and becomes free-swimming. Irrespective of whether or not the protoplast becomes a zoospore, development into a colony is by the same sequence of plakeal stages as in asexual reproduction. The colony thus formed consists of but one or two hundred cells. This colony always reproduces asexually and its gonidia produce colonies with a somewhat larger number of cells. Reproduction for a half dozen or more generations is also exclusively asexual, and in each succeeding generation there are a somewhat larger number of cells.

Ulothrix

Uninucleate filamentous green algae with a parietal chloroplast constitute the Order Ulotrichales, of which *Ulothrix* is a member.

The systematic position of *Ulothrix* (after R.E. Lee, 2008) is as follows:

Domain: Eukaryota
Kingdom: Viridiplantae
Phylum: Chlorophyta
Class: Ulvophyceae
Order: Ulotrichales
Family: Ulotrichaceae
Genus: *Ulothrix*

Ulothrix is a genus of filamentous green algae, generally found in quiet or running freshwater and occasionally on wet rocks or soil. They thrive in the low temperatures of spring and winter. The thallus consists of unbranched filaments of indefinite length that are affixed to the substratum by a special basal cell called the **holdfast cell**. Its cells are normally as broad as they are long.

Reproduction

Ulothrix reproduces by the following three methods:

1. Asexual by vegetative mode
2. Asexual by sporulative mode
3. Sexual mode

Asexual by vegetative mode

It occurs by fragmentation which may be accidental, due to the movement of animals or by water currents especially in running or falling waters. It takes place naturally by a change in pH or temperature or during drying of the habitat or by death or emptying of some intervening cell. The fragments may rise to the surface and form floating filaments or develop rhizoidal cells for attachment to the substratum.

Asexual by sporulative mode

It takes place by the formation of motile or non-motile spores (mitospores) of the following types:

Zoospores: They are formed during favourable periods, where all the cells, except holdfast, function as zoosporangia. In other words, all of the cells except the basal one are capable of cell division and forming zoospores. Zoospore formation can also be induced artificially by removing the filament from flowing to still water. In many northern lakes in the temperate parts of the world, *Ulothrix zonata* grows abundantly in early spring in shallow waters along rocky shorelines. *Ulothrix zonata* is dominant until the water temperature reaches 10°C, when it disappears owing to massive conversion of the thallus to zoospores. Thus, slight elevation in temperature also favours zoospore production. In culture, formation of the zoospores of *Ulothrix zonata* occurs around 20°C at relatively high light levels and photoperiods of either short-day (8 hours light : 16 hours dark) or long-day cycles (16 hours light : 8 hours dark). Zoospore formation is minimal at 5°C, low irradiance and neutral day length (12 hours light: 12 hours dark) (Graham and Krantzfelder, 1986).

In most cases, *Ulothrix* forms quadriflagellate zoospores (Fig. 1).

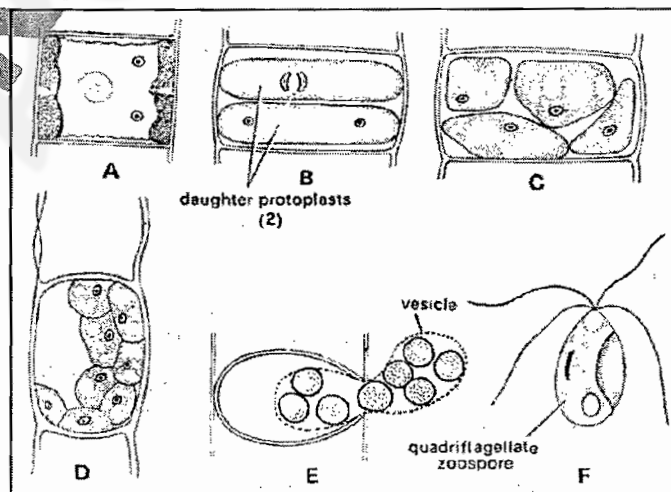


Figure 1: Zoospore production in *Ulothrix*

Species with narrow cells form 1, 2, or 4 quadriflagellate zoospores per cell, whereas those with broad cells form 2, 4, 8, 16, or 32 zoospores per cell. The zoospores have a conspicuous eyespot and are liberated through a pore in the side of the parent wall. Zoospores from species with narrow filaments are the same size, whereas those from broad-celled species form two types of zoospores, namely macro- and microzoospores that differ from each other in size, position of the eyespot, and length of the swarming period.

West in 1904 reported biflagellate zoospores in *Ulothrix* sp., which is now considered doubtful. However, in classical Phycology, it has been generally agreed that *Ulothrix* produces three types of zoospores (Fig. 2):

1. Macrozoospores with four flagella
2. Microzoospores with four flagella
3. Microzoospores with two flagella

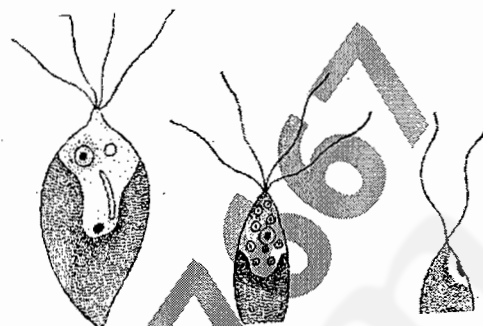


Figure 2: Different types of Zoospores in *Ulothrix*

The formation of Zoospores has an apical- basal gradient that is it starts in the terminal part and descends downwardly. The process of zoospore formation progresses as follows:

1. The zoosporangia grow in size and develop a food reserve. After a while, the protoplast contracts from the cell wall.
2. The contracted protoplast undergoes successive mitotic divisions. The chloroplast and pyrenoids divide with the division of the protoplast. Thus 1-32 daughter protoplasts are formed in a single zoosporangium.
3. All the daughter protoplasts get metamorphosed into flagellate zoospores. They are liberated through a lateral pore which develops by localized gelatinization of the cell wall. All the zoospores are released in a thin hyaline and gelatinous vesicle.
4. The vesicle dissolves or bursts a little later to liberate the zoospores. This event takes place generally just after sunrise. If the plants are kept in dark conditions, the zoospores do not come out (Stephan, 1998; Zavahir, J. S. and Seneviratne, G. 2007).
5. A mature zoospore is ovoid in shape and lacks a cell wall. It is green and motile structure as it mostly bears four whiplash flagella. There are 3 morphological types of zoospores distinguishable.
 - a. Macro zoospores, which are of large size with four anterior flagella. They are produced 1-8 per cell. During germination they come to rest with help of their posterior pointed end.
 - b. Tetra flagellates micro zoospores, which are relatively smaller. Usually, 4-32 of them are formed per cell. They are ovoid or pyriform. They also bear four anterior flagella.
 - c. Biflagellate micro zoospores: They are biflagellate pyriform zoospores produced very rarely. As mentioned earlier, its existence is now considered doubtful (Lechene *et al*, 2007).

Germination of Zoospores: After completion of the swarming period a zoospore settles down on some solid object. Microzoospores attach themselves to the substratum by their anterior end while macrozoospores do so by their pointed posterior end. It then loses its flagella, stigma and contractile vacuoles. A thin wall is secreted around it. The attaching end fits into the minute crevices of the substratum like a drop of jelly. The germling elongates at both the attached and free ends. Soon it undergoes divisions into two cells. The lower cell develops into holdfast or rhizoidal cell. The other cell remains green and forms the green part of the filament.

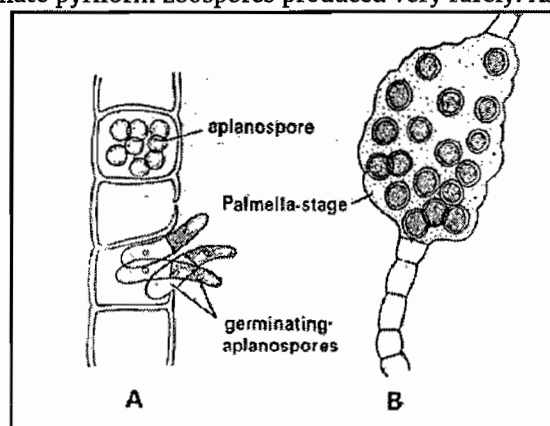


Figure 3: A. Aplanospores & B. Palmella stage in *Ulothrix*

Aplanospores & hypnospores are formed (Fig. 3) when the contents of a sporangium do not escape as motile zoospores but become transformed into one or more non-motile spores. They possess a thin or thick wall. Accordingly they are known as aplanospores (thin-walled) and hypnospores (thick walled). These spores may remain green or become brown. **palmella stage** is commonly formed on the damp banks of water (Fig. 3). The cell walls gelatinize to produce a mucilaginous envelope around the partially naked cells. These cells divide in all directions and give rise to a number of daughter cells. The walls of the daughter cells also undergo gelatinization. In this way a large number of non-motile, green and almost naked cells come to lie in a cover of mucilage. It is known as palmella or palmelloid stage. Palmella stage is a device to protect the cells against desiccation since mucilage retains water for a long time.

The mucilage dissolves on being flooded. The liberated palmella spores can germinate directly or can get transformed into zoospores.

Sexual mode of reproduction

Gametes of *Ulothrix* are formed in the same way as zoospores but are biflagellate. As with the zoospores, all of the cells except the basal one are capable of cell division and forming the gametes. Gamete production occurs towards the end of growing season & under longer photoperiods (Lokhurst, 1974). The protoplast of a gametangium divides by mitosis to produce 64 or even 128 biflagellate gametes. The gametes are small, green & naked structures.

In *Ulothrix* species, the gametes are of the same size, with fusion occurring only between gametes from different filaments. Thus sexual fusion is isogamous but heterothallic (Lee, 2008). However, homothallism & anisogamy has been reported rarely in *U. rorida*.

Swimming gametes get attracted to each other following the chemotaxis generated by a Ca^{++} containing phero-hormone (Williamson & Ashley, 2002) and later fuse from their lateral sides to form a diploid zygote (Fig. 4).

There is never any parthenogenetic development of unfused gametes (Cardinale *et al*, 2007). Earlier, it was believed that parthenogenesis is found in *U. flacca* during short day light conditions.

The zygote has four flagella, two eye spots, four contractile vacuoles and two chloroplasts. The zygote remains motile for a while, settles, secretes a thick wall, and undergoes a resting period during which it accumulates a large amount of storage material. The first division of the zygote is meiotic, with the zygote forming 4 to 16 zoospores or aplanospores (Berger-Perrot *et al*, 1993).

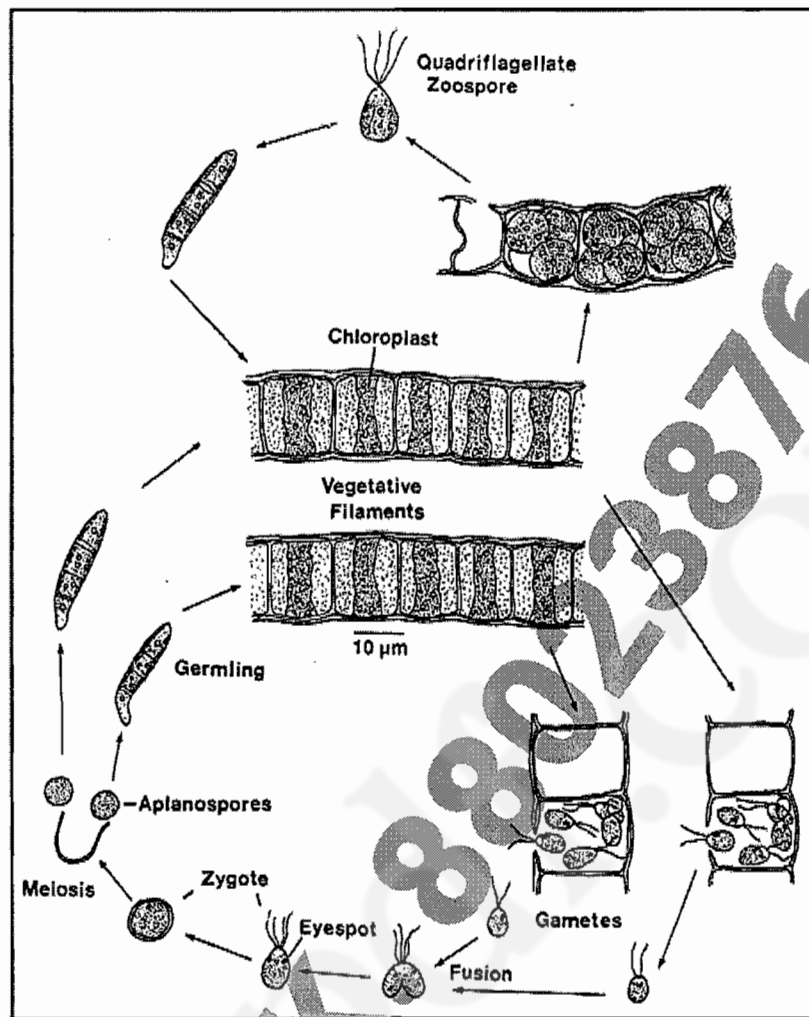


Figure 4: Life Cycle of *Ulothrix* (also showing the sexual process)

Origin of sex as understood from *Ulothrix*

Ulothrix normally reproduces by two types of flagellate structures, zoospores and gametes. Zoospores are formed during favourable season of growth while gametes are produced towards the end of growing season. When growth and development are slowed down zoospores even if formed, will not be having enough food nor will they be sufficiently active to give rise to new filaments. On account of the lack of active locomotion, these swimmers tended to come together. Fusion of two swimmers might have occurred in the remote past, which resulted in the reactivation of the fusion protoplast due to the pooling of resources and genetic potentialities of the two. The diploid structure also developed the mode of perennation. Since the benefits accruing out of this accidental fusion were profound, it is now widely believed that the act came to stay in the life history of algae and plants. The process is described in Figure 5 below.

Several evidences are shown by *Ulothrix* to prove that the gametes are modified zoospores. Some of them are:

1. Both the zoospores and gametes are formed inside similar type of cells in *Ulothrix* by very similar processes.
2. Formation of these swimmers shows apical-basal gradient.
3. Except for the tendency for fusion, the gamete is in every way similar to the biflagellate micro zoospore.
4. Recent works done on the motile cells of *Ulothrix* (Williamson & Ashley, 2002) suggest many biochemical similarities, such as large deposits of Calcium Alanine.

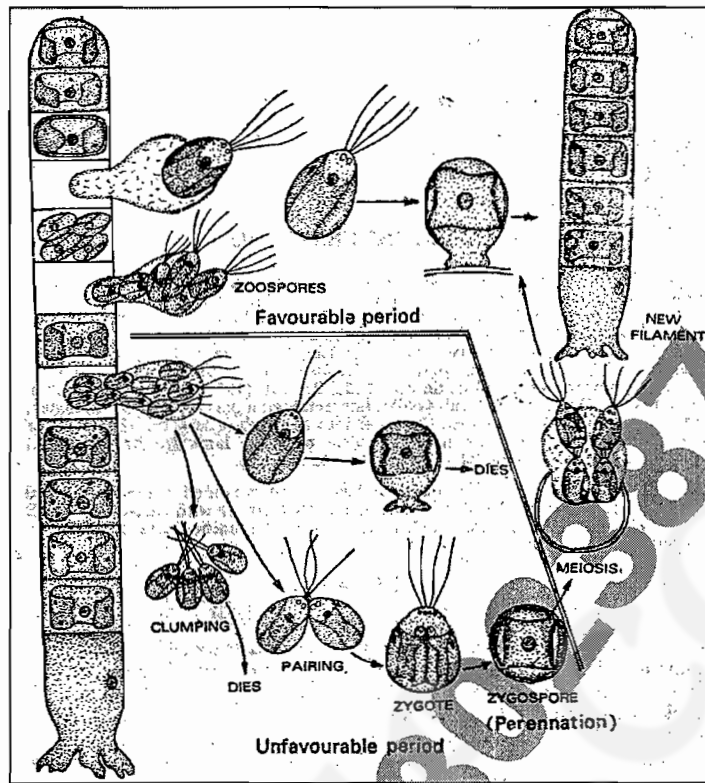


Figure 5: Reproductive transition in *Ulothrix*

Ulva: An Account of Life Cycle

About the Genus

Ulva is a genus of the class Ulvophyceae in Chlorophyta.

It commonly known as 'sea-lettuce' or 'green laver', is exclusively a marine green alga, usually found attached to rocks in intertidal zones of the sea.

Ulva species have thalli with expanded blades two cells thick (distromatic). *Ulva* are parenchymatous: cell division may occur anywhere on the thallus but always in a plane perpendicular to the thallus surface. Compared to more advanced algae and vascular plants, their construction is relatively simple. They do not differentiate into tissue layers or show much specialization among cells. The cells themselves are irregularly arranged and are quadrate. The cell walls are fibrillar and made up of cellulose. They store energy as starch. Arranged in sheets only two cells thick, *Ulva*'s large surface to volume ratio allows it to have a high nutrient uptake.

Ulva can also occur as a hollow cylinder. Such morphological types were previously considered under the genus *Enteromorpha*. However, these thalli composed of hollow cylinders are now recognized as species of *Ulva* (Hayden et al., 2003). The change from tubular form to expanded blade form is possible, with help of a morphogenetic substance called *Thallusin*. Bacteria of the Cytophaga-Flavobacterium-Bacteroides group grow on the surface of the algae and produce this morphogenetic factor called thallusin (Matsuo et al., 2005). The alga absorbs thallusin, resulting in the familiar morphology of the thallus.

Reproduction and Life Cycle

Ulva sp. reproduces by the following three methods:

1. Vegetative
2. Asexual
3. Sexual

1. Vegetative reproduction

Vegetative multiplication usually takes place by fragmentation. Any part of the thallus, if detached accidentally or towards the old age, may regenerate into new thallus. It occurs quite commonly in species growing in quiet waters of estuaries. But, species growing in open hardly show any vegetative multiplication.

2. Asexual reproduction

The asexual reproduction takes place by *quadriflagellate zoospores*, produced in sporophytic thallus [2n]. Any vegetative cell of the thallus may act as zoosporangium. But, zoospores are first formed in the cells near the margin and later in almost all other cells of the thallus. At the time of zoospore formation, the protoplast of the vegetative cell divides repeated mitoses to form 4-8 daughter protoplasts. The first division is a reduction division, so the daughter protoplasts are haploid structures. Each haploid daughter protoplast metamorphoses into a uninucleate, quadriflagellate zoospore.

Zoospores are usually released in the morning when the thallus is wet by the water of incoming tides. They swarm for an hour or so, and then come to rest on some suitable substratum, withdraw their flagella and germinate to produce haploid gametophytic thalli.

3. Sexual reproduction

The sexual reproduction is usually strictly *heterothallic & isogamous*. It takes place through gametes which develop in the cells of haploid gametophytic [n] thallus. Reproductive areas are formed near the margins of the thallus and the fertile portions change from green to olive-green to brownish-green. Any cell of the fertile region can act as gametangium. Thus, we see a similarity in the initiation of zoosporogenesis & gametogenesis. Yet, there are profound differences in both the processes, most remarkably in the process of cell division itself. The gametogenic protoplast divides repeatedly by *mitosis* to form 32-64 biflagellate gametes.

The gametes are positively phototactic. Each has a chloroplast with a pyrenoid and an eye spot.

Some species of *Ulva* like *U. zabala* have been reported to be anisogamous. In these species, it is possible to distinguish between male and female gametophytes by differences in the colour of the fertile portion. Furthermore, the male gametes are narrower and smaller with a yellowish green chloroplast and indistinct pyrenoid, whereas the female gametes are larger with a bright green chloroplast and distinct pyrenoid.

Gametes are usually liberated in the morning, at the beginning of a series of spring tides. Two gametes from two different thalli fuse to form a diploid quadriflagellate zygote. The zygote swarms for a short while, and then comes to rest, loses its flagella, and secretes a wall. Within a few days, it divides by a mitotic division. Out of the two daughter cells thus formed, one develops into a rhizoid and the other into a blade. Thus the sporophytic thallus [2n] is formed by the germination of the zygote.

In some species such as *U. crispata*, parthenogenetic development of the gamete gives rise to a new thallus. There is a periodic reproductive pattern in *Ulva*, controlled by the lunar cycle.

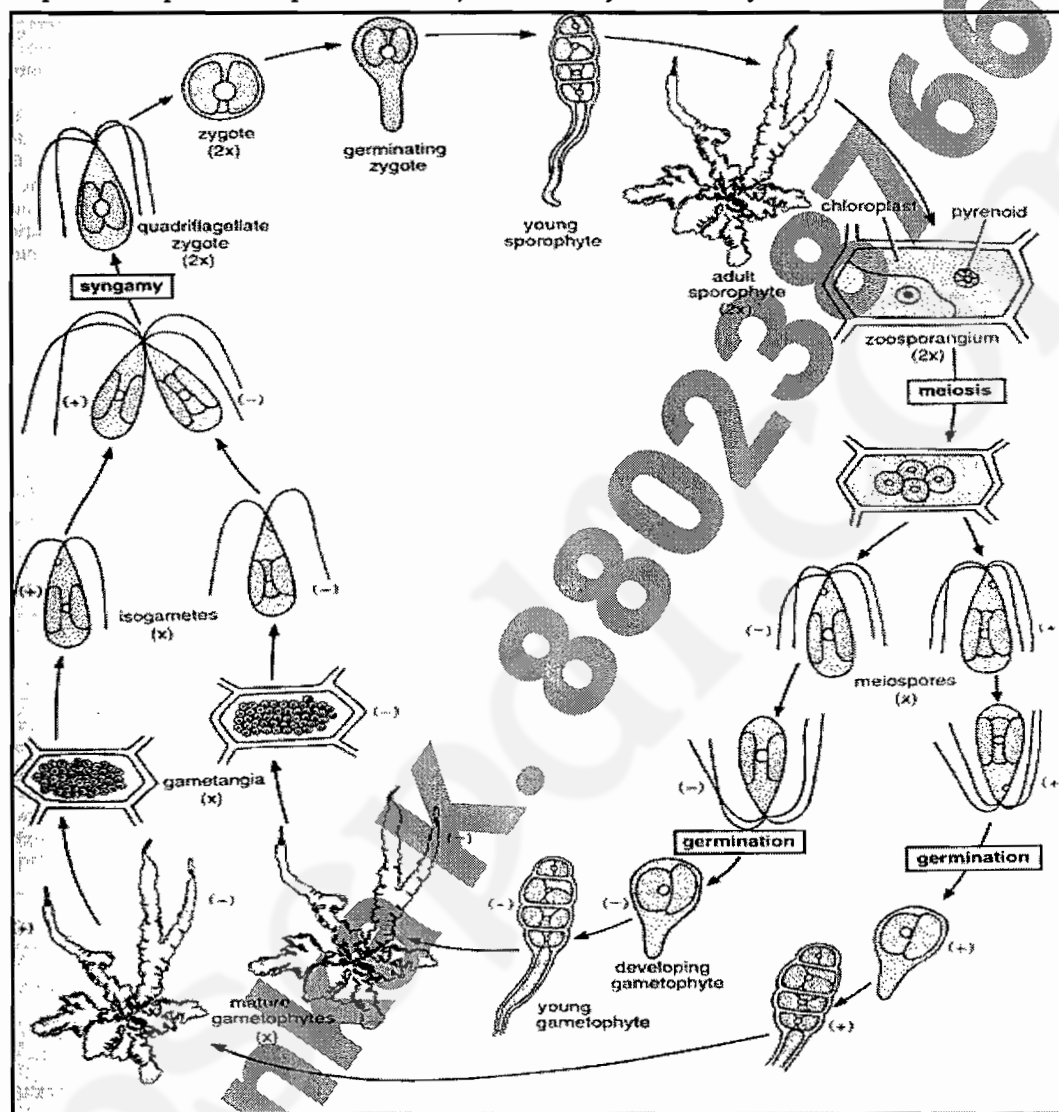


Figure 1: Life Cycle of *Ulva*

Spirogyra: An account of Reproduction

About the Genus

1. *Spirogyra* is strictly a **freshwater filamentous** alga, found as **free-floating** tangled masses.
2. The filaments are simple and unbranched consisting of a row of cylindrical cells arranged end to end. The cells are longer than broad. All the cells are alike and so, there is **no distinction** into base or apex.
3. The cells have **cellulose cell wall**, **differentiated in primary and secondary walls**.
4. The cell wall is covered with mucilage sheath composed of **calcium pectate & hemicelluloses** which make the filament slimy to touch.
5. The protoplast of each cell contains cytoplasm, a single nucleus and generally one **characteristically ribbon shaped chloroplast** with numerous **pyrenoids** embedded in it.
6. All cells of the filament are capable of cell division.

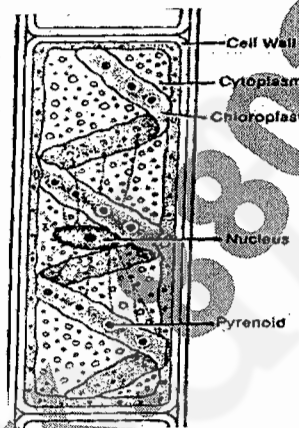


Figure 1: A Single Cell of *Spirogyra*

Reproduction

In *Spirogyra*, multiplication takes place by vegetative or sexual methods of reproduction. **Asexual reproduction is completely absent.**

Vegetative reproduction- Long filaments of *Spirogyra*, while floating, get broken up into small parts or fragments by accident. Each piece grows into a new *Spirogyra* filament. This is known as fragmentation.

Sexual reproduction- Sexual reproduction takes place by means of **conjugation**. There are basically two types of conjugations seen in *Spirogyra* spp.

Scalariform conjugation The conjugation, taking place between two cells of opposite filaments. It is very common in most of the species of *Spirogyra*.

Lateral conjugation takes place between two adjacent cells of the same filament. It is not so common, but in *S. affinis* and *S. tenuissima* it is almost a rule.

1. Scalariform Conjugation

During scalariform conjugation, the filaments lie parallel to each other in common mucilage. At many points of proximity, opposite cells produce peg-like outgrowths. They produce tube like structures, which soon come in intimate physical contact with each other. Later, the end walls at the point of contact dissolve, forming a passage between the opposite cells. Thus, a **conjugation tube** is formed.

Meanwhile, the entire protoplast of each cell shrinks and is rounded up into a **single non-flagellate gamete**. One of the gametes moves from its cell into the opposite one by an **amoeboid movement**. The migrating gamete is the - type or the male one and the other stationary gamete is the + type or the female

one, the cells forming them being called as male or female or – and + types respectively. The male gamete, after moving into the female cell, fuses with its gamete forming the zygote.

2. Lateral conjugation.

In this type, a lateral protrusion is produced by cell of a filament towards another cell present immediately next in the same filament. Once the lateral peg comes in physical contact with the neighbouring cell, the septum dissolves and a passage is established between adjacent cells. Male gamete moves into the female cell through the conjugation tube and fuses with its gamete, forming the zygote. After lateral conjugation, a female cell having a zygote is always found to be adjacent to an empty male cell.

Nakahara [1992] suggested that zygnematalean conjugation might have been derived from oogamy, in response to the selective pressures operating in shallow waters.

Zygote

The Zygote, which is a product of sexual fusion & hence a diploid structure, stores food [especially a large amount of fat] and develops a thick wall to cross over the unfavourable period.

It germinates when conditions are good. To restore the haploid condition of the vegetative phase, it undergoes meiosis prior to germination. This results in four haploid nuclei, but only one survives and the others degenerate. The outer thick wall of the zygote bursts and the inner layer grows into a long tube, which undergoes transverse division into two cells. The lower cell contains scanty chlorophyll and it develops into a rhizoid, which remains in the zygospore membrane for some time and degenerates later. The upper cell continues to divide so that a new

Spirogyra filament is formed.

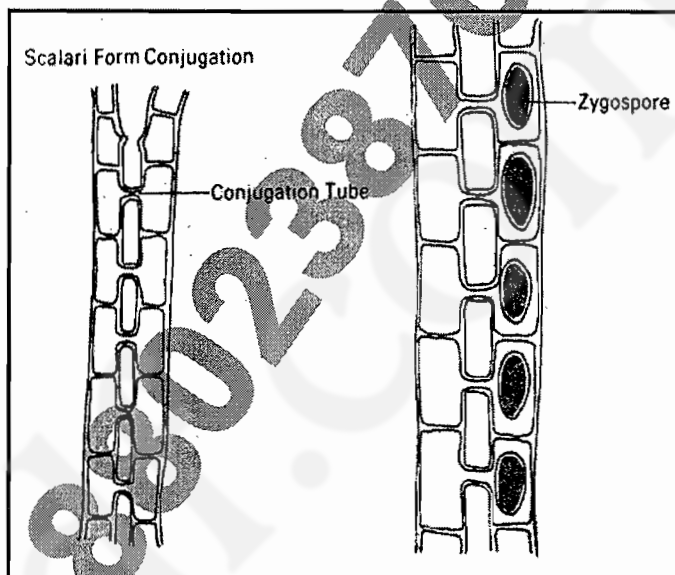


Figure 2: The Scalariform Conjugation in *Spirogyra* sp

Oedogonium: An account of Reproduction

About the Genus

Oedogonium is a member of the Chlorophyta with the following systematic position (R.E. Lee, 2008):

Division: Chlorophyta

Class: Chlorophyceae

Order: Oedogoniales

Family: Oedogoniaceae

The members of the order Oedogoniales are uninucleate filamentous freshwater algae with a unique type of cell division; motile spores and gametes with a whorl of flagella at one pole. Sexual reproduction is oogamous, and asexual reproduction can be by zoospores or akinetes.

Chloroplasts in this order are reticulate, extending from one end of the cell to the other.

The Oedogoniales and its single family, the Oedogoniaceae, have only three genera—*Oedogonium*, *Oedocladium* and *Bulbochaete*. *Oedogonium* is unbranched, whereas *Oedocladium* and *Bulbochaete* are branched.

These algae are usually present in permanent bodies of water such as ponds or lakes. If they are growing in moving water, they are seldom in the fruiting condition. Normally fruiting takes place in the summertime.

Cell Division

Cell division involves the breaking of the parent wall and the formation of apical caps. In *Oedogonium*, cell division (Fig.1) is initiated by the formation of a ring under the wall in the upper part of the cell (Hill and Machlis, 1968). The ring enlarges by the coalescence of material produced in the cytoplasm. While the ring is being produced, the nucleus migrates to the center of the cell and divides mitotically. During late telophase, the new cross wall begins to form by means of a phycoplast. The daughter cells elongate, causing a split in the parent wall near the apical ring. This rupture in the cell wall is caused by the material in the apical ring, which expands as the cells elongate. Each daughter cell eventually elongates to about the same length as the mother cell, elongation being completed within 15 minutes. The material of the ring becomes the cuticle, and a new cell wall is laid down under it.

During the elongation of the daughter cells, the new transverse wall moves up to the base of the newly formed secondary wall and fuses with it.

Cell division is intercalary in *Oedogonium*, therefore division of every cell in the filament and repeated division of the daughter cells result in alternate cells with and without caps of old cell walls. This theoretical condition usually does not occur in nature. Repeated division of the distal daughter cell commonly results in filaments in which a cell with an apical cap is successively followed by several cells without caps.

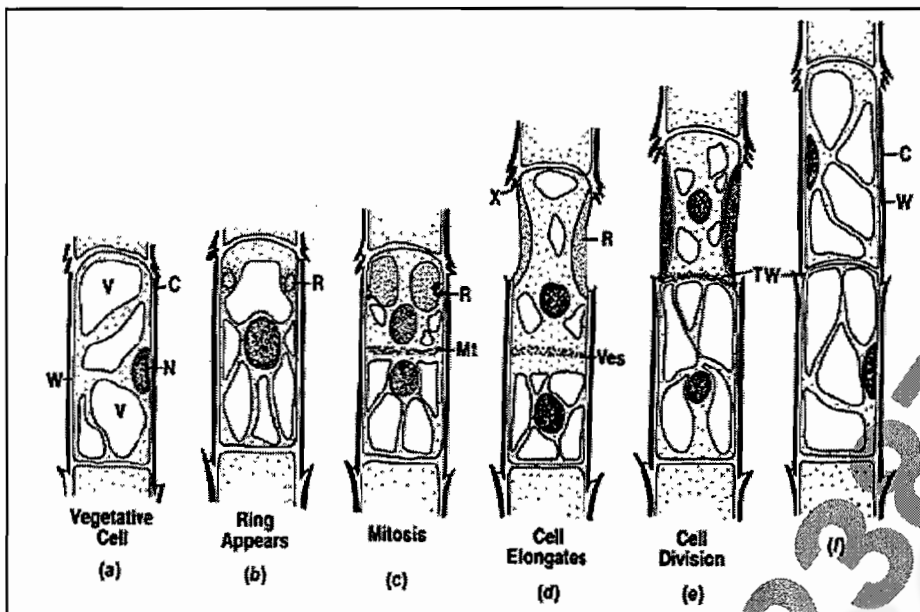


Figure 1: Cell division in *Oedogonium*. (C) cuticle; (Mt) microtubules; (N) nucleus; (R) ring; (TW) transverse wall; (V) vacuole; (Ves) vesicles; (W) wall; (X) cap. (After Hill and Machlis, 1968)

Asexual Reproduction

Asexual reproduction is by means of zoospores. Zoospores are formed singly within a cell and usually only in those cells with apical caps. The earliest sign of zoosporogenesis is the appearance of a small electron-dense mass in an invagination of the nuclear envelope (Pickett-Heaps, 1971). From this the centrioles appear and multiply rapidly, forming two adjacent rows near the nucleus. The nucleus, with its two rows of centrioles, moves to the lateral wall. The two rows of centrioles move apart in the center to form a circle of centrioles under the plasmalemma. The centrioles extrude the flagella and the flagella roots.

The Golgi secretes a fibrillar hyaline layer around the zoospore, which makes up the vesicle in which the zoospore is initially encased. The Golgi also secretes mucilage to the base of the zoospore, which probably aids in extrusion of the zoospore. The lateral wall of the parent cell splits at the apical cap, and the zoospore in a vesicle emerges through the aperture. The vesicle opens later, releasing the zoospore. The zoospore has about 30 flagella linked together by a striated root at the apical end (Hoffman and Manton, 1962); it swims for about an hour, settles, retracts its flagella, and develops a holdfast that attaches to the substrata. This then develops into a new filament.

Aplanospores can also be formed, and resemble oogonia.

Sexual Reproduction

Sexual reproduction is oogamous and, depending on the behavior of the male filaments, either *macrandrous* if the male filament forms the sperm directly, or *nannandrous* if the sperm are produced in a special dwarf male filament (Fig. 2).

In many species of *Oedogonium*, it is relatively easy to induce sexual reproduction by placing old filaments in fresh media and saturating the atmosphere with carbon dioxide. Nitrogen limitation has also been reported to induce the formation of oogonia (Singh and Chaudhary, 1990).

Development of antheridia In macrandrous species, the oogonia and antheridia may develop on the same filament (homothallic or monoecious species) or on two different filaments (heterothallic or dioecious species). The antheridia are produced by the division of antheridial mother cell. This cell divides into two unequal cells. In this division, the upper cell is much shorter than the lower. The lower cell then divides repeatedly to produce a series of 2 to 40 antheridia. Each antheridium forms two sperm, which are liberated in a vesicle by transverse splitting of the wall. The sperm then escape from the vesicle. The sperm are similar in structure to the zoospores but smaller and more elongated with a crown of about 30 flagella (Hoffman and Manton, 1963), and are yellowish-green because of their reduced plastids. In a macrandrous species such as *Oedogonium cardiacum* (Hoffman, 1960), the sperm are attracted to the oogonium by a chemotactic substance secreted by the oogonium.

In nannandrous species, the antheridia are produced on special dwarf males or nannandria, majority of which are heterothallic (dioecious). These 'dwarf males' or nannandria originate by germination of a special type of swarmer known as **androspores** produced singly in flat cells, i.e. androsporangia formed by repeated transverse divisions of the ordinary vegetative cells. The androsporangia may occur on the same filaments on which the oogonia are produced or on different filaments. The androspores resemble in shape and structure to zoospores. They are somewhat smaller than zoospores and larger than antherozoids. After their small swarming period, they settle down either on the oogonium or on one of the neighbouring cells. Here the androspore germinates and develops into a dwarf male. The nannandrium consists of one basal cell attached to the oogonium or adjacent cell, and one or more flat antheridia.

Development and structure of oogonia In both macrandrous and nannandrous species the oogonia develop by terminal or intercalary oogonial mother cells. The oogonial mother cell divides transversely and the upper daughter cell always develops into an oogonium, which always possesses one or more caps at its upper end. The lower daughter cell is called the 'suffultory cell'.

On maturity the oogonium is rounded or ovoid with a hyaline spot near the upper end. It contains a large spherical egg cell in it.

Fertilization The swimming antherozoid approaches the oogonium at the receptive (hyaline) spot. The flagella are retracted and the plasmogamy takes place, which is soon followed by karyogamy. The zygote or oospore which is somewhat retracted from the oogonial wall secretes a three-layered thick smooth wall around it.

Germination of zygote The zygote liberates once the oogonial wall decays. Prior to germination the zygote undergoes a period of rest, which ranges from 12 to 14 months. During the resting period, there is meiotic division, which restores the haploid nucleus of the alga's vegetative stage. The haploid protoplast divides and four daughter cells are developed. Each of them metamorphoses in a zoospore. The growth of these zoospores follows the same pattern as the vegetatively produced zoospores.

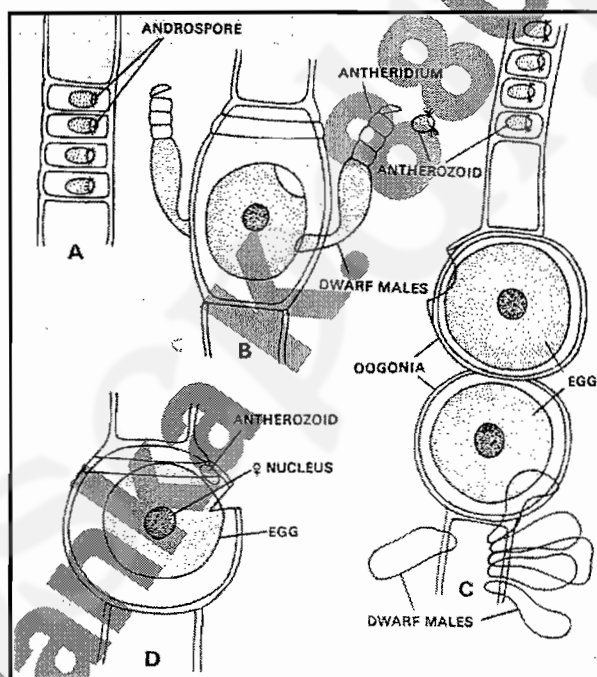


Figure 2: Nannandrous sexual behavior in *Oedogonium*

Chara: An account of Reproduction

About the Genus

Chara is a member of the Chlorophyta with the following systematic position (R.E. Lee, 2008):

Division: Chlorophyta

Class: Charophyceae

Order: Charales

Family: Characeae

The members of the order Charales, have large, macroscopic, thalli growing up to 120cm long. They are multicellular, heterotrichous and branched and show oogamous sexual reproduction with sterile cells surrounding the sex organs. Zoospores are never formed by the members of the Charales. The sperm cells produced by the members of this order are similar to the flagellated male gamete of the Bryophytes and many Pteridophytes.

The vegetative body is complex showing advanced characters like:

1. Plasmodesmatal connections between adjacent cells
2. Apical growth
3. Differentiation between nodes and internodes

It is now universally accepted that it is the line of algal evolution that led to the development of the land plants. There are numerous evidences supporting this view. Because of this fact and also due to an advanced type of construction, the vegetative body of Charalean members is called plant, a term which is no longer applied to most other algae (Marin & Melkonian, 1999).

The members of Charales grow in fresh water. Many of the Charales are heavily calcified, with concentrations of plants on the bottom of lakes leading to the formation of marl (CaCO_3 and MgCO_3 deposits) and hence the common name of the Charales, **stoneworts**. There are about 400 species of Charales world-wide.

Reproduction in Chara

The reproduction in *Chara* spp. takes place by vegetative and sexual methods. Asexual reproduction by zoospore formation is not found.

1. Vegetative reproduction.

The vegetative reproduction takes place by

1. Bulbils and tubers
2. Amylum stars
3. Secondary protonema.

By tubers and bulbils- The bulbil are commonly formed on rhizoids or sometimes even on buried nodes. The whole structure is full of starch. Sometimes the globule divides and becomes multicellular and known as 'simple tuber'. When the tuber appears on the node, some of the peripheral cells go on dividing and massive structure is developed. Each starch filled tuber may develop into a separate plant.

By amyllum or starch stars- The cells of some subterranean nodes become star-shaped and very much laid in by starch are called amyllum stars. Each such structure develops into a new plant.

By secondary protonema- The protonemata like filamentous outgrowths come out from a node or rhizoid. Each such outgrowth is capable to develop into a new plant.

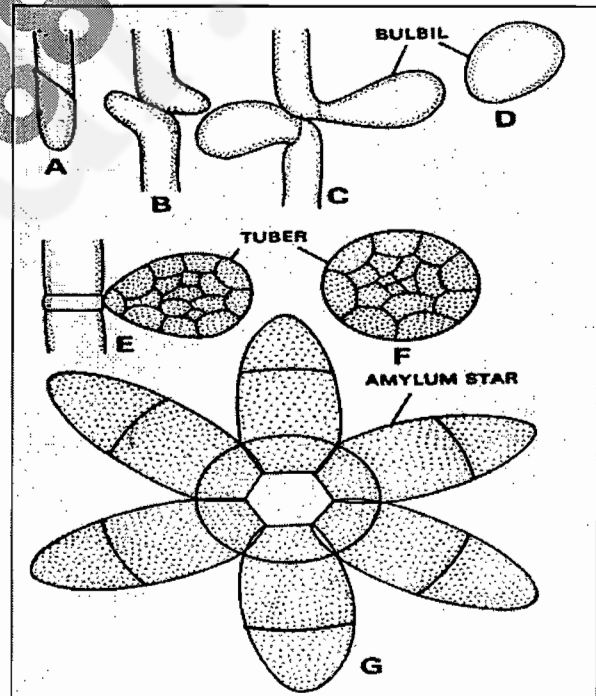


Figure 1: Some vegetative propagules in *Chara*. A-D: Formation of Bulbil; E-F: Formation of Tuber; G: Amyllum Star

2. Sexual reproduction

The sexual reproduction is oogamous. A very advanced and specialized type of oogamy is found and there is a special terminology for the sex organs. The male fruiting body is called globule and the female nucule.

Most of the species are homothallic (monoecious). Globules and nucules are borne on nodes, usually on the same node. In *Chara*, the nucule is above the globule. One globule and one nucule borne on one node.

Development of globule– A single superficial nodal cell of the adaxial side of the branch acts as the initial of both the fructifications, i.e., nucule and globule. This superficial cell divides into two derivatives by a transverse wall. One cell derivative is the initial cell of the globule and the other is the initial cell of the nucule.

The globule initial cell divides transversely and two daughter cells are formed. The lower daughter cell does not divide further and converts into the *pedicel cell*. The upper daughter cell divides twice successively by longitudinal divisions in planes perpendicular to each other. After this, four cells are formed arranged in quadrants. Each of these quadrants divides transversely to produce eight cells with their apices joined in the center of the sphere (Octant Stage). Two successive periclinal mitotic divisions produce three cells within each cell of the octant yielding a sphere composed of 24 cells.

The outermost eight cells are called **shield cells**. The middle cells are known as **manubrial cells** and the innermost eight cells are **primary capitulum cells**. The shield cells become very much enlarged and expanded. They form the colorful cover of the globule due to chromoplasts near the inner wall of the cells. The color changes from pale yellow to orange as the globules mature. The outer walls of the shield cells fold inward and the shield cells appear multicellular structures. The infoldings are incomplete. The manubrial cells become very much radially elongated, but the primary capitulum cells are arranged compactly to each other in the centre of the globule.

From each primary capitulum cell six secondary capitulum cells are cut off inside the globule. These secondary capitulum cells rarely develop tertiary cells. The secondary capitulum or the tertiary capitulum cells (if present) cells give rise to the **antheridial filaments**. Each antheridial filament has many compartments or cells in it. Each cell of an antheridial filament is an **antheridium** whose protoplast matures into a single **antherozoid**. The antherozoid is coiled in a compact helix of two and a half turns in the antheridium.

When the antherozoids are mature, the shield cells of the globule separate from one another and expose the antheridial filaments. Soon after the antheridial filaments are exposed, and antherozoids emerge backward through a pore in the cell wall.

Liberation of antherozoids generally takes place in the morning, and their swarming may continue until evening (Smith, 1955). The sperm have scaly cell wall, two somewhat unequal flagella attached sub-terminally near the anterior end of the cell.

Development of nucule– The nucule develops adjacent to the globule, just above it.

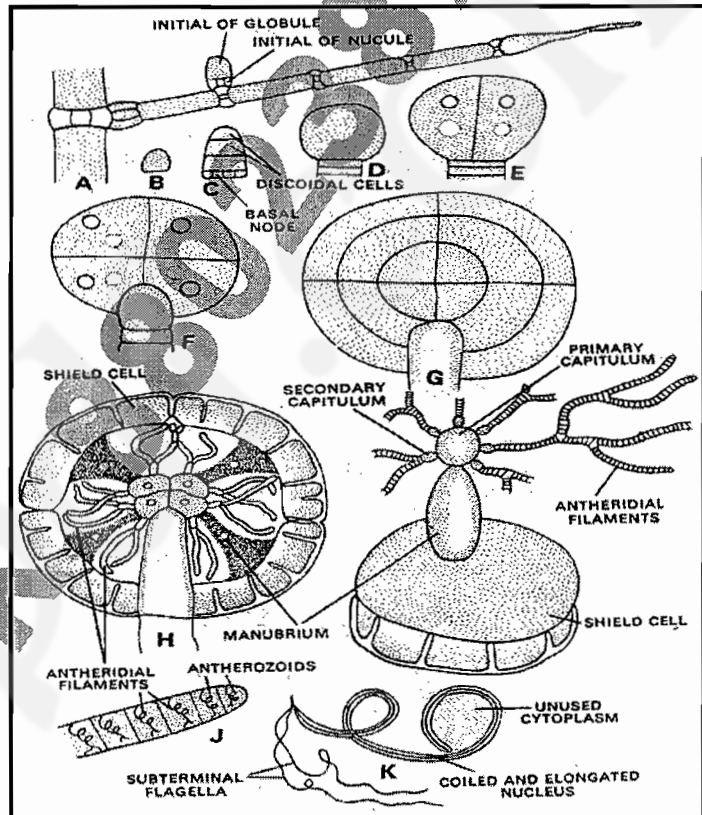


Figure 2: Development of Globule in *Chara*

The nucule initial divides twice and a row of three cells is formed. The terminal cell acts as oogonial mother cell which elongates sufficiently in vertical direction and transverse wall develops in the lower region of it dividing it into two cells. The lower small cell and the upper one is oogonium, which contains an egg. The lowermost cell of the row of three cells does not divide and acts as a pedicel. The middle cell divides vertically in such a way so that a single central cell and five sheath initials are produced. The sheath initials surround the central cell. The sheath initials elongate vertically sometimes even before the vertical elongation of the oogonial mother cell and encircle it. Each of the sheath initials divides transversely forming the upper tier of **corona cells** and lower tier of **tube cells**. The tube cells elongate several times to their original length and become spirally coiled around the oogonium. The corona cells do not elongate much and act collectively as the **corona** of the nucule.

Fertilization- Prior to fertilization the elongated and twisted tube cells become separated from each other and five small slits are developed just below the corona. The swimming antherozoids around the nucule try to enter through these openings.

The flagella are withdrawn and one of the antherozoids penetrates the egg. The male nucleus travels downwards and fuses with the egg nucleus developing a diploid (2n) nucleus. This diploid nucleus shifts in the bottom of the zygote. The zygote settles down in the mud, secretes a thick wall and germinates on the approach of favourable conditions.

In favourable conditions, the zygote germinates by dividing meiotically producing four haploid nuclei. Simultaneously a septum divides the zygote into two unequal cells. The small distal cell is **lenticular cell** and contains one functional nucleus in it. The remaining big cell is called **storage cell**, possessing three nuclei in it which disintegrate very soon. The outer wall of the ornamented zygote cracks and the lenticular cell exposes. The lenticular cell divides by a vertical wall giving rise to a protonematal initial and a rhizoidal initial. The protonematal initial develops into a primary protonema, which later on differentiates into nodes and internodes. The rhizoidal initial gives rise to a colourless rhizoid having nodes and internodes.

The life cycle is of haplontic type. All phases but zygote are haploid.

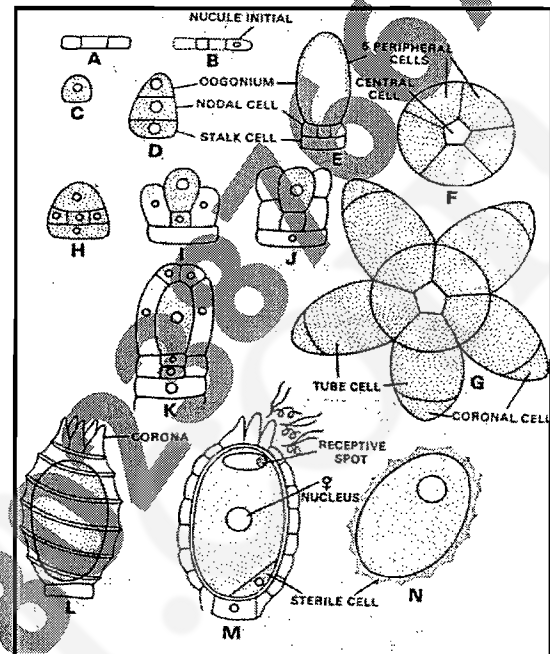


Figure 3: Nucule Development in Chara

Advanced features of Charales

The position of the Charales is considered more advanced than the rest of the green algae because of several reasons, most important of which include the following.

1. They have the multicellular female organ surrounded by a sheath like structure.
2. They have the complex antheridia surrounded by several sterile cells.
3. They show strong apical growth by vegetative shoots. Growth occurs by a dome-shaped apical cell, each derivative of the apical cell dividing transversely into two daughter cells. The upper daughter cell is the nodal initial; and the lower is the internodal initial. The nodal initial matures into the cells of the node, and the internodal initial matures into the single long cell of the internode (Smith, 1955). The specialized apical growth superficially resembles that of the Equisetales. However, the Charales are devoid of vascular system and they cannot be compared with Equisetales.
4. Their plant body has a degree of specialization which is generally not found in other green algae. The algae in the Charales have an axis divided into nodes and internodes. Each node bears a whorl of branches composed of a number of cells.
5. The male gametes have a cell covering of scales.
6. Like the land plants, no zoospores are formed.
7. Like the land plants, a phragmoplast develops during cell division, resulting in the formation of a cross wall with plasmodesmata.
8. The motile cells are similar to the flagellated male gametes of the bryophytes and vascular cryptogams. These motile cells are asymmetrical and have two laterally or subapically inserted flagella.

9. The microtubular root system contains a multilayered structure that is associated with a broad microtubular root and a second, smaller, microtubular root.
10. Rhizoplasts are not present.
11. The mitotic spindle is persistent during cytokinesis.
12. No eye-spots occur.
13. Like the land plants, glycolate is broken down by glycolate oxidase, whereas urea is broken down by urease.

It is now generally agreed that this is the line of algal evolution that led to the development of land plants. The land plants (embryophytes) probably evolved from algae similar to those in the Charales (Karol et al., 2001; Lee 2008). As such, the Charales are sometimes separated from the rest of the green algae and grouped with embryophytes as streptophytes (Lewis and McCourt, 2004).

Coleochaete: An Account of Life Cycle

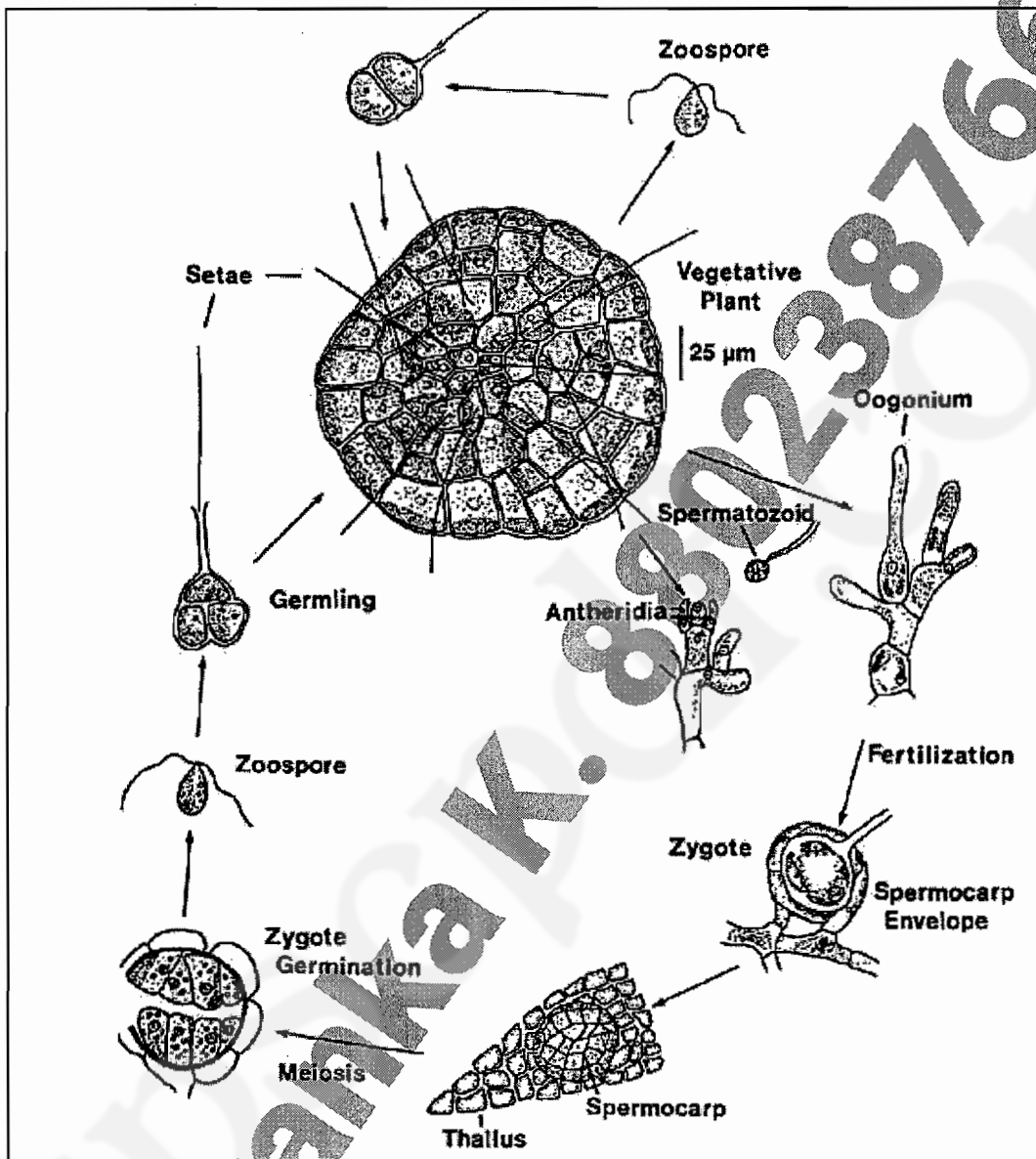


Figure 1: Life Cycle of *Coleochaete*

About the Genus

Coleochaete is a genus of the order Coleochaetales in Chlorophyta (Lee, 2008).

The algae in the Coleochaetales are characterized by the presence of sheathed setae and by oogamous sexual reproduction. Small-subunit ribosomal RNA sequencing (Wilcox et al., 1993; Kranz et al., 1995) has indicated that the green algae in this order evolved into the bryophytes and lycopods.

Most of *Coleochaete* sp. occur as epiphytes or endophytes in the shallow littoral zone of freshwater lakes (Ciminio and Delwiche, 2002).

The thallus is composed of flat sheet of cells (in *C. scutata*) or branching filaments of cells (in *C. pulvinata*).

All members of the genus have sheathed hairs called *setae*. The base of the *setae* is covered with a gelatinous material. The *setae* probably function as an antiherbivore defense, since broken *setae* exude a substance that repels potential predators (Marchant, 1977).

Reproduction

Asexual reproduction is by means of biflagellate zoospores that are formed singly within a cell. Zoospores and spermatozooids of *Coleochaete* are asymmetrical, are covered with scales, and possess multilayered structures and micro-tubular roots similar to those of lower land plants (Graham and McBride, 1979; Sluiman, 1983). The flagella arise subapically and project to the side of the cell.

Isolated cells of a thallus may produce zoospores at any time of the year, but in the spring there is frequently a production of zoospores by every cell in a plant living over from the previous summer. A zoospore escapes by moving in an amoeboid manner through a pore in the parent-cell wall and then swims for an hour or so before it comes to rest and secretes a wall. The one-celled germling soon begins to develop into a multicellular thallus, and, when a developing thallus consists of a few cells only, the cellular arrangement is that characteristic of the species. One or more cells of young developmental stages bear *setae*. Aplanospores with fairly thick walls may also be developed singly within a cell.

Sexual Mode: Most species of *Coleochaete* also reproduce sexually which is oogamous and, according to the species, the plants are heterothallic or homothallic. There are dwarf species that form zoospores only. In *C. pulvinata* the antheridia are bluntly conical and are usually borne at the tips of branches. Antheridia of *C. scutata* are developed midway between center and periphery of the discoid thallus. In this species a vegetative cell divides into two daughter cells, one of which, the antheridial mother cell, redivides to form antheridia. Antheridia of *Coleochaete* each produce a single biflagellate antherozoid which may be green or colourless.

Oogonia of *C. pulvinata* are formed by a metamorphosis of one-celled lateral branchlets. The oogonium of this species is a flask shaped structure with a long colorless neck, the trichogyne. Oogonia of *C. scutata* have an inconspicuous trichogyne. They are formed from marginal cells of a thallus.

Fertilization takes place by an antherozoid swimming into an oogonium and there uniting with the egg. The zygote remains within the oogonium, secretes a thick wall, and increases greatly in size. At the same time there is an upgrowth of branches from the cell below the oogonium and from neighboring cells to form a parenchymatous layer that more or less completely encloses the oogonium. The oogonium with its ensheathing layer of cells, which soon become reddish-brown, is termed a **spermocarp**. The spermocarps remain dormant over winter. The gametes uniting to form a zygote have nuclei of quite different size. But the male gamete nucleus increases greatly in size as it approaches the female nucleus, and, when the two fuse, they are of approximately the same size.

Germination: Division of the zygote nucleus is meiotic to restore the haploid state of the vegetative stage. Mitotic division follows the reductional division until there are 8 to 32 daughter protoplasts and then each of them is metamorphosed into a biflagellate zoospore. The zoospores are liberated by a breaking of the spermocarpic and zygote walls. The liberated zoospores swarm for a short time and then come to rest and develop directly into new thalli.

Phaeophyceae

Introductory Notes and General Characters

The Phaeophyceae, or brown algae, constitute a class within the algal phylum Heterokontophyta (Lee, 2008). Their important features are as follows.

1. The members derive their characteristic colour from the large amounts of the carotenoid fucoxanthin ($C_{40}H_{56}O_6$) in their chloroplasts as well as from any phaeophycean tannins that might be present.
2. The chloroplasts also have chlorophylls *a*, *c*₁ and *c*₂.
3. There are two membranes of chloroplast E.R., which are usually continuous with the outer membrane of the nuclear envelope.
4. The storage product is laminarin. However, the accumulation product of photosynthesis is D-mannitol, a sugar alcohol.
5. There are no unicellular or colonial organisms in the order, and the algae are basically filamentous, pseudoparenchymatous, or parenchymatous.
6. The brown algal structure ranges from simple to complex & very large parenchymatous forms.
 - a. Heterotrichous forms, e.g., *Ectocarpus*
 - b. Multiaxial construction forms, e.g., *Myriactula pulvinata*
 - c. Uniaxial pseudo-parenchymatous forms, e.g., *Hecatonema sargassicola*
 - d. Parenchymatous forms, e.g., *Laminaria*, *Fucus*.
 - e. *Postelsia palmaeformis* assumes the form of a miniature tree and is commonly known as sea palm.
7. Many species possess air bladders which keep them afloat.
8. Growth of the thallus may be apical, intercalary or trichothallic.
9. Phaeophycean cell wall has two layers.
 - a. The outer mucilaginous layer has alginic and fucinic acid. Alginic acid is of commercial importance and is obtained from *Laminaria*, *Sargassum* and *Ascomyllum*. It is used in the manufacture of adhesives and artificial silk.
 - b. The inner layer is mainly cellulosic.
10. The cytoplasm contains many refractive bodies called fucosan vesicles. They are abundant in the cytoplasm of metabolically active cells.
11. The cells are uninucleate with one or more nucleolus. In some brown algae (e.g., *Fucus*) the resting nuclei show chromocentres which are not found in other groups of algae.
12. The flagellated structures have a pair of laterally inserted unequal flagella, of which the larger one is tinsel type and faces forward, whereas the smaller one is generally whiplash and faces backward.
13. They reproduce by vegetative, asexual and sexual methods. Except for *Tilopteridales*, *Dictyotales* and *Fucales*, all brown algae reproduce asexually by zoospores.
14. In most brown algae fertilization is external, i.e. gametes fuse outside the gametangium in water. There is no reduction division in zygote; it forms a diploid thallus on germination.
15. The life cycle of brown algae may have isomorphic or heteromorphic type of alternation of generations, or it can be simply diplontic.
16. They are found almost exclusively in the marine habitat, there being only four genera containing freshwater species, that is, *Heribaudiella*, *Pleurocladia*, *Bodanella*, and *Sphacelaria* (Schloesser and Blum, 1980). A number of marine forms penetrate into brackish water, where they often form an important part of the salt marsh flora. These brackish water algae have almost totally lost the ability to reproduce sexually, and propagate by vegetative means only.
17. Most of the Phaeophyceae grow in the intertidal belt and the upper littoral region. They dominate these regions in colder waters, particularly in the Northern Hemisphere, where the number of phaeophycean species is less than that of the Rhodophyceae, but the number of phaeophycean population is much greater. In the tropics, the only place where large numbers of Phaeophyceae are found is the Sargasso Sea of the Atlantic. In India brown algae are commonly found on the western and southern coasts.

18. The Phaeophyceae probably evolved from an organism in the *Phaeothamniophyceae*, which have motile cells similar to those in the Phaeophyceae, but lack the characteristic unilocular and plurilocular sporangia of the Phaeophyceae

Classification

Fritsch (1935), on the basis of vegetative structure and sexual reproduction, divided the class Phaeophyceae into nine orders: (1) Ectocarpales, (2) Tilopteridales, (3) Cutleriales, (4) Sporochneales, (5) Desmarestiales, (6) Laminariales, (7) Sphacelariales, (8) Dictyotales, and (9) Fucales.

Smith (1955) recognized the following three classes and 12 orders in the division Phaeophyta:

1. **Isogeneratae:** Members of this class are characterized by alternation of two isomorphic generations. It has five orders differing in vegetative structure, mode of growth and structure of reproductive organs: (1) Ectocarpales, (2) Sphacelariales, (3) Tilopteridales, (4) Cutleriales, (5) Dictyotales.
2. **Heterogeneratae:** Those brown algae which show alternation of heteromorphic (i.e., morphologically distinct) generations are included in this class. It has six orders: (1) Chordariales, (2) Sporochneales, (3) Desmarestiales, (4) Punctariales, (5) Dictyo siphonales, (6) Laminariales.
3. **Cyclosporeae:** The brown algae included in this class have a Diplontic life cycle in which there is no alternation of generations. This class includes only a single order, Fucales.

R.E. Lee in 2008, identified 7 orders of Phaeophyceae. The orders considered by him are presented in an evolutionary sequence with the Dictyotales and Sphacelariales being the most ancient and the Ectocarpales and the Laminariales the most recent.

Order 1 Dictyotales: growth by an apical cell; meiosis occurring in the production of four to eight non-motile spores; oogamous sexual reproduction.

Order 2 Sphacelariales: growth by an apical cell; daughter cells divided longitudinally to give a polysiphonous structure; isogamous sexual reproduction.

Order 3 Cutleriales: trichothallic growth forming a fan-like thallus in at least one generation; anisogamous sexual reproduction.

Order 4 Desmarestiales: trichothallic growth forming axial cells; oogamous sexual reproduction.

Order 5 Ectocarpales: thallus consisting of filaments or filaments compacted together; reproduction isogamous or anisogamous.

Order 6 Laminariales: diploid thallus parenchymatous resulting from an intercalary meristem between the stipe and blade; reproduction oogamous.

Order 7 Fucales: growth primarily by a promeristem; gametophyte reduced to egg and sperm; oogamous sexual reproduction.

Fucus: An Account of Life Cycle

An Introduction to the Order Fucales

The Fucales constitute an advanced Order in the class Phaeophyceae of Heterokontophyta. The Fucales are usually distributed in the colder waters of the world.

Its members are characterized by the following features.

1. The organisms in this order are parenchymatous with growth from an apical cell.
2. The haploid generation is reduced to the egg and sperm, with the remainder of the life cycle being diploid.
3. The gametes are borne in special cavities, the **conceptacles**.
4. Conceptacles may be scattered over the surface of the thallus, but more frequently they are limited to the inflated tips of special branches, the **receptacles**.
5. Gametic union is always oogamous.
6. The Fucales are worldwide in distribution, but those of the Arctic and north temperate seas differ considerably from those of the Antarctic and south temperate waters. *Fucus* is a common genus in northern waters, whereas in tropical and subtropical waters *Sargassum*.

Morphology and Anatomy

The thallus is much branched and is supported by a short narrow stalk that is attached to a discoid holdfast.

Each branch has an apical cell at its apex. The apical cell divides several times a year, resulting in the formation of a dichotomy or fork, with one arm of the fork being longer than the other. The apical cell in mature *Fucus* is a four-sided pyramid with a flattened base.

The branching is dichotomous, with each flattened segment having a prominent central midrib surrounded on both sides by a narrower wing. The wings usually bear scattered cryptoblasts, which are basically sterile conceptacles with large numbers of hairs that facilitate the uptake of nutrients from the seawater (Hurd et al., 1993). At certain times of the year, the tips of the branches are swollen into receptacles that contain the fertile conceptacles. The inflation of the receptacles is due to the production of a large amount of mucilage.

Life Cycle

Fucus does not show asexual reproduction. The life cycle is diplontic type. The gametes represent the only haploid stage of the life cycle.

The gametes are borne in conceptacles (Figs. 1) that are similar to the cryptoblasts except that the colorless hairs are restricted to a small area near the aperture.

In sexual behaviour, some species of *Fucus* are dioecious while others are monoecious. A conceptacle usually contains just one kind of sex organ, but sometimes both types of the sex organs are found within a single conceptacle.

Each conceptacle is developed from a single superficial cell which lies close to the apical cell of the receptacle. At first, the initial cell of a conceptacle lies at the same level with the adjoining surface cell, but as adjoining cells divide and re-divide the conceptacle initial comes to lie below the thallus surface.

The initial divides transversely. The outer daughter cell, the *tongue cell*, does not divide again and eventually disappears. The inner daughter cell, the *basal cell*, divides and redivides to form a layer, the fertile sheet, two or three cells in thickness. The fertile sheet is the layer lining the flask-shaped conceptacle. A mature conceptacle is globose and with a relatively small opening, the *ostiole*.

Superficial cells of the fertile layer may produce multicellular and branched filaments (*paraphyses*) or they may produce sex organs.

Filaments developed in the upper portion of a conceptacle are unbranched, do not bear sex organs, and project through the ostiole and a very short distance beyond it. These filaments are called *periphyses*.

There are two possible ways for antheridial development. They may develop directly from cells of the fertile layer, or develop at the base of branched paraphyses.

Division of the primary nucleus of an antheridium is meiotic, and after meiosis is completed there are mitotic divisions that continue until there are 64 nuclei.

Nuclear division is followed by a cleavage of the antheridial contents into uninucleate protoplasts which metamorphose into biflagellate antherozoids. Antherozoids of *Fucus*, similar to those of other Fucales, have

an anteriorly pointed tinsel flagellum and a whiplash posterior flagellum. The wall of an antheridium is differentiated into an outer firm layer, the *exochite* and an inner more gelatinous layer, the *endochite*.

Superficial cells of the fertile layer function as initial cells of oogonia. An oogonial initial divides transversely into a *stalk cell* and an *oogonium*, neither of which divides again.

The oogonium increases greatly in size. Its nucleus divides meiotically and the four nuclei formed by meiosis divide once to form eight nuclei.

There is then a cleavage of the oogonial protoplast into eight uninucleate eggs. All species of *Fucus* form eight functional egg cells. This a characteristic feature of the genus.

The wall of an oogonium consists of three concentric layers: *exochite*, *mesochite*, and *endochite*.

Liberation of antherozoids and eggs generally takes place when *Fucus* is flooded by the incoming tide. Imbibition of water by the oogonial mesochite and endochite causes them to swell, burst the exochite, and slip out of it.

The eight eggs, still surrounded by the two inner wall layers, are then passively pushed between the paraphyses till the ostiole, and then are pushed out through between the periphyses. This passive extrusion of eggs and the surrounding wall layers seems to be due to a swelling of gelatinous material within the conceptacle which exerts a pushing force.

After being extruded from a conceptacle, the endochite imbibes more water and its swelling causes the mesochite to rupture apically and expose the endochite.

As the exposed endochite continues to imbibe water and become softer the mutually compressed eggs become rounded, separate from one another, and eventually float away from one another through the watery endochite.

Re-flooding of a thallus also causes a rupture of the exochite of an antheridium and an extrusion of the contained mass of antherozoids through the ostiole.

The mass of antherozoids is still surrounded by the endochite when first extruded, but this soon gelatinizes and the antherozoids swim freely in all directions.

The antherozoids cluster in such numbers around each egg as to cause it to rotate in the water.

The sperm are attracted to the eggs by a species-non-specific pheromone, *fucoserraten*, released by the eggs (Müller and Jaenicke, 1973). The species-specific recognition between eggs and sperm is based on specific oligosaccharides on the eggs and sperm. The oligosaccharide side chains of the egg-surface glycoproteins contain fucosyl, mannosyl, and/or glucosyl residues (Wright et al., 1995).

The glyco-proteins on the sperm eventually bind to the complementary glycoproteins on the egg. These results in two "blocks" to further sperm penetration (Wright et al., 1995):

1. A "fast block" within seconds caused by depolarization of the plasma membrane due to Na^+ and Ca^{++} influx. Excess sperm detach from the egg following depolarization.
2. A "slow block" results from the formation of a cell wall around the zygote by the release of cellulose, phenolics, sulfated fucans, vanadate peroxidase, and alginates from cortical vesicles to form the adhesive glycocalyx.

Due to these mechanisms polyspermy is prevented in *Fucus* species.

Within less than an hour after fertilization the zygote forms a gelatinous wall that firmly affixes it to any substratum upon which it has lodged.

In less than a day the zygote shows a definite polarity, as is evidenced by the sending out of a rhizoidal protuberance. External factors affecting the polarity include: gradients in light (both visible and ultraviolet 2. temperature, 3. Hydrogen-ion concentration and 4. Proximity of other zygotes. The establishment of polarity due to the effect of auxin, a substance which has been shown to be present in eggs of *Fucus*.

The embryo divides following a species specific pattern. Eventually a multicellular embryo with an apical cell is differentiated.

This embryonal structure develops to give rise to a diploid sporophytic thallus and completes the life cycle.

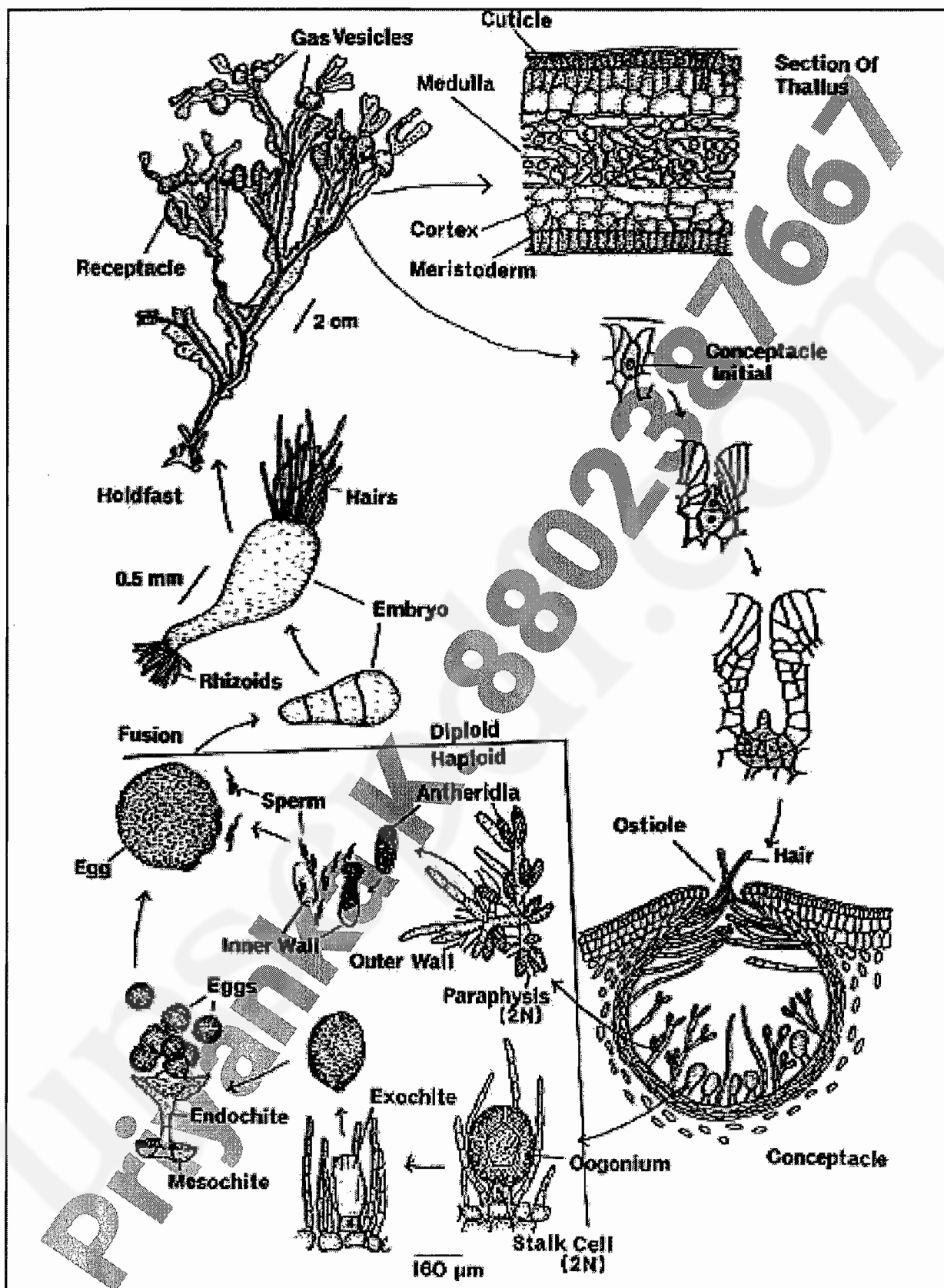


Figure 1: Life Cycle of *Fucus*

Laminaria: An Account of Reproduction

An Introduction to the Order Laminariales

The Laminariales constitute an advanced Order in the class Phaeophyceae of Heterokontophyta. The Laminariales are very large algae that are usually distributed in the colder waters of the world.

Its members are characterized by the following features.

1. The members of this order have parenchymatous thallus.
2. The members have an alternation of a large sporophyte with a microscopic gametophyte. Many of the genera have sporophytes that produce upto 40 metres large thallus. Such forms are regarded as Giant Kelps. The sporophytes are differentiated into a holdfast, stipe, and blade.
3. The thallus growth is from an intercalary meristem between the stipe and blade.
4. The sporophyte has some degree of tissue differentiation. There are three different tissues in the sporophyte: the central medulla, the cortex, and the epidermis (Fig 1). The haptera lacks a medulla, but all three tissues are present in the stipe and blade. The stipe and blade have the same anatomy, the only difference being the cylindrical to elliptical shape of the stipe and the flattened shape of the blade.
5. Sexual reproduction is oogamous.
6. With the exception of *Chorda* and *Saccorhiza*, the Laminariales lack an eyespot and an associated flagellar swelling in the motile cells.

Life cycle of Laminaria

The life cycle of a member of the Laminariales involves the alternation of a large sporophyte with a microscopic gametophyte (Fig. 2)

The sporophyte produces haploid zoospores by reduction division. The reduction divisions occur in unilocular sporangia. These sporangia develop near the margins of the blade. The sporangia are organized into reproductive sori. The sporangial development occurs late during sporophyte development. The intercalary meristem between the stipe and the blade secretes chemicals that travel distally to inhibit the formation of reproductive sori on the sporophyte (Luning et al., 2000). The production of the inhibitory chemicals is greatest during the rapid growth in the first part of the year. In the second part of the growing season, activity of the intercalary meristem and growth slows down resulting in decreased production of the chemical inhibitors. After this, the formation of sori occurs on the sporophyte.

The sori contain unilocular sporangia intermingled with paraphyses. The upper end of the paraphysis is swollen and mucilaginous. Thirty-two haploid zoospores are formed in the unilocular sporangium (Motomura et al., 1997), and the zoospores are released through the thickened apex of the sporangium.

The zoospores have a single chloroplast. The zoospores are positively chemotactic towards nutrients (Amsler and Neushel, 1989) and can be transported for several kilometers (Reed et al., 1988) during the 48 hours of swimming. The zoospores contain glycoproteins in small vesicles in the peripheral cytoplasm that are released when the zoospores settle. These glycoproteins adhere the cells to the substratum. The settled zoospore secretes a thin wall around itself, with a slender germ tube emerging.

After settling, the zoospores produce the gametophytes, which are of microscopic dimensions. The gametophytes are dioecious, with separate male and female thalli.

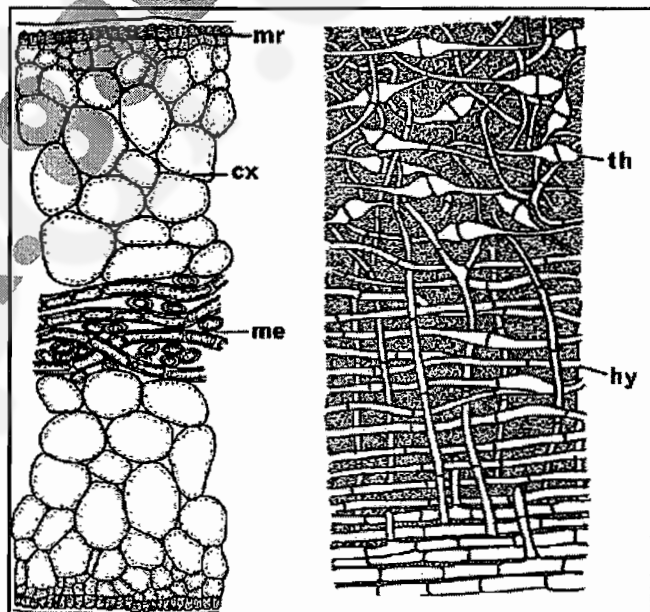


Figure 1: Sections of lamina (left) and the central portion of a stipe (right) of a member of the Laminariales. (cx) Cortex; (hy) hyphae; (me) medulla; (mr) meristoderm; (th) trumpet hyphae.

In *Laminaria*, Evans (1965) showed that there is an X/Y sex-determining mechanism, with segregation taking place at the meiotic division in the unilocular sporangium.

The male gametophyte has smaller cells and is more branched than the female. The male gametophytes produce small colorless antheridia.

In the female gametophyte, elongated oogonia are formed that produce a single egg.

Under long-day conditions (16 hours light: 8 hours dark), eggs are released during the dark cycle, mostly during the first 30 minutes of darkness (Lüning, 1981). The release is controlled by a circadian rhythm. After the female cell has emerged, the thick plastic edges of the wall contract and form a platform on which the egg remains for some time. The sexual hormone lamoxirene is secreted by the eggs. The sexual hormones ectocarpene and desmarestene are also present, but they do not have hormonal activity in *Laminaria*.

Spermatozoids are ejected from the antheridia within a few seconds of exposure to lamoxirene. The spermatozoids are attracted to the eggs where fertilization takes place. The zygote germinates to form a flat proembryo that subsequently develops into the mature sporophyte.

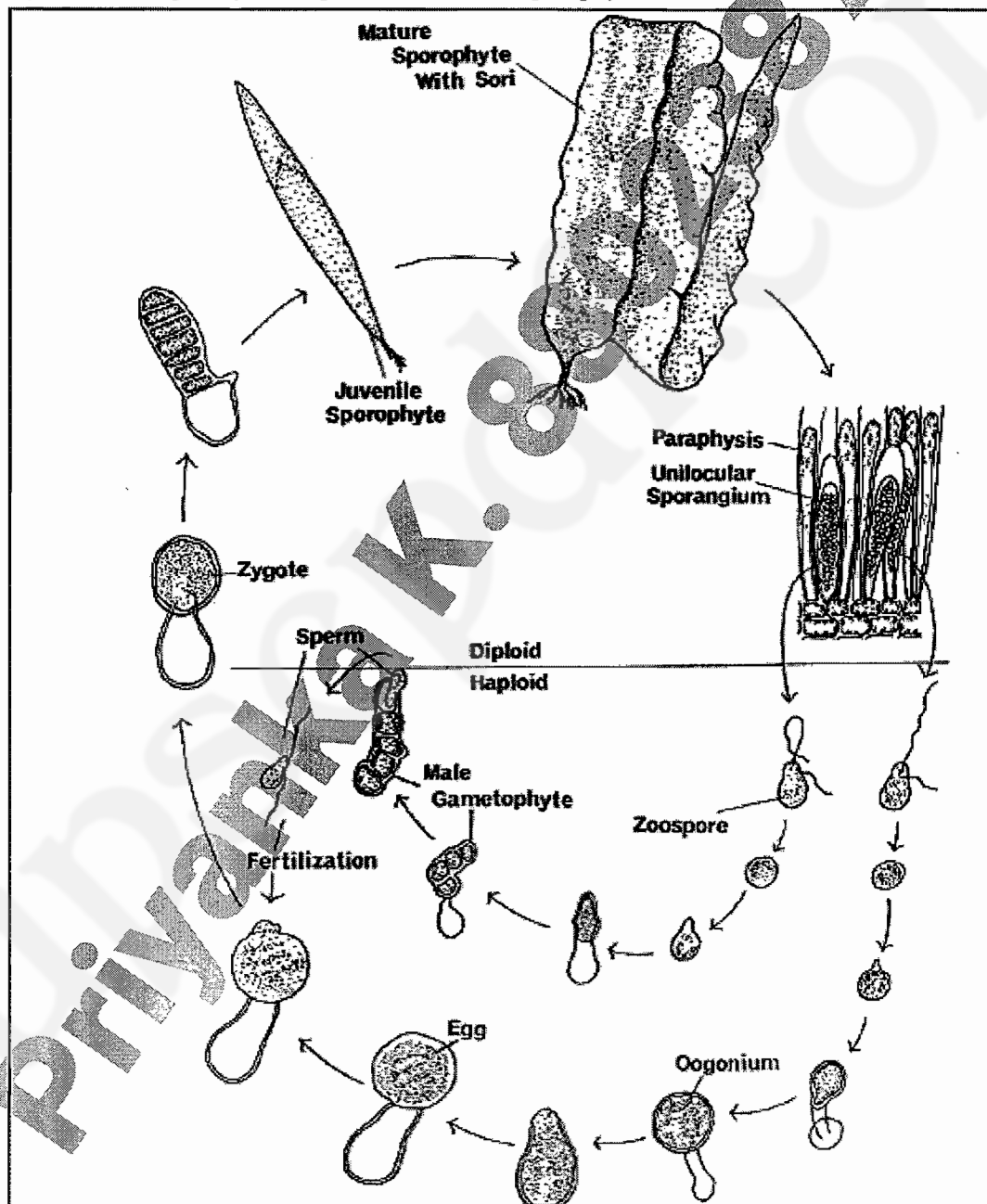


Figure 2: Life Cycle of *Laminaria japonicum*

Ectocarpus: An Account of Reproduction

An Introduction to the Order Ectocarpales

1. Almost all the 60 genera the order Ectocarpales are found in the cold sea water of the temperate and polar regions.
2. Most of the members of this order are heterotrichous with a prostrate creeping disc, which functions as holdfast and an erect monosiphonous filamentous foliose system.
3. These algae show trichothallic growth with intercalary divisions confined to certain areas in the filament.
4. Asexual reproduction is by zoospores which are produced in unilocular or plurilocular sporangia.
5. Sexual reproduction is isogamous but in some species it may be oogamous. Reproductive structures are borne laterally or in uniseriate rows.
6. Modern algologists restrict Ectocarpales to a single family Ectocarpaceae characterized by simple, heterotrichous members exhibiting isogamous sexual reproduction and isomorphic alternation of generations.

Ectocarpus Life Cycle

Ectocarpus shows a life cycle with isomorphic alternation of generations, where a diploid thallus alternates with a haploid thallus in the life cycle. Both the alternating thalli would be alike, except for their reproductive behaviour.

The thallus of *Ectocarpus*, be it gametophytic or sporophytic, is heterotrichous,

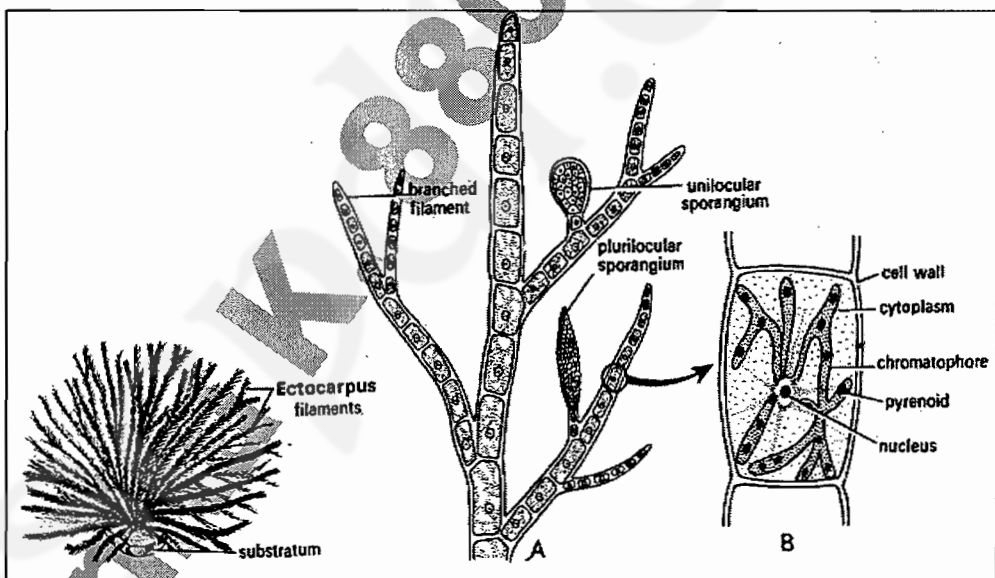


Figure 1: Structure of *Ectocarpus*

which means that it is differentiated into a prostrate rhizoidal system and an erect branched system. The prostrate system is branched, and attaches the thallus to the substratum which in some species may be a supporting algal body.

The erect part of the thallus is usually irregularly branched. The filaments are several centimeters long and monosiphonous i.e. only one cell in thickness. In some species (e.g., *E. granulosus*) the basal parts of the main erect filaments become corticated by rhizoidal branches. The distal part of each filament terminates into mucilaginous hairs.

The prostrate part of *Ectocarpus* thallus shows apical growth, whereas it is intercalary in the erect part.

Ectocarpus reproduces by asexual and sexual methods in an alternating way.

Asexual Reproduction

Asexual reproduction takes place in the sporophytic (diploid) algal body by biflagellate zoospores, where two dissimilar flagella are inserted laterally in a typical pheophycian way.

The sporophytic (diploid) thallus produces two types of sporangia:

1. unilocular zoosporangia, which form haploid zoospores
2. plurilocular or neutral zoosporangia, forming diploid zoospores.

Both of these types of zoosporangia are borne on the same algal body or on different algal bodies.

Unilocular sporangia

They develop singly at the tips of small branchlets by cellular enlargement with accompanying sporangial initial. The first division of diploid nucleus is meiotic and it is followed by mitotic divisions. This results in the formation of 32 or 64 haploid nuclei.

Each nucleus enveloped by a small protoplasmic segment metamorphoses into a pyriform, uninucleate, biflagellate zoospore. The two flagella are laterally inserted and are unequal; the anterior is longer and tinsel type and the posterior is shorter and whiplash type.

The Zoospores are discharged in the morning, like most other algae. The zoospores ooze out of the sporangium in a gelatinous matrix through a small terminal perforation. Soon after their discharge they become free and swim off individually for less than 30 minutes. They withdraw their flagella and attach themselves to the substratum by their anterior ends. They germinate within 2-3 hours and produce haploid filaments.

Plurilocular or neutral sporangia

These arise very much like the unilocular sporangia from terminal cells of the branchlets of diploid thalli. However, the sporangial initial in this case divides mitotically and repeatedly in transverse and vertical planes, forming as many as 660 cubical cells arranged in definite tiers. This multicellular structure is known as plurilocular sporangium. Each diploid uninucleate cell of the sporangium metamorphoses into a single diploid biflagellate zoospore, which is morphologically alike to but genetically distinct from the zoospore formed by the unilocular sporangium, which is haploid.

These zoospore remain motile for a longer period of time i.e. 3-5 hours, which may be because of their better chances of survival conferred by diploidy (Vonhertiz, 1997). They germinate to form diploid asexual thalli. They do not play any part in alternation of generation, hence termed Neutral Spores by classical algologists.

The formation of unilocular and plurilocular sporangia by a species is affected by temperature and salinity of water. For example, *E. siliculosus* produces unilocular sporangia at 13°C, plurilocular at 19°C and both unilocular and plurilocular at 16°C. Rozzet et al. (2000) have discovered an elaborate signaling process in *Ectocarpus* that couples the environmental conditions to specific zoospores production by selective gene transcription via the Transcription Factor activation route.

Sexual Reproduction

Most of the species of *Ectocarpus* are anisogamous in sexual reproduction, although some may be isogamous or oogamous. The anisogamy may be physiological (e.g., *E. siliculosus*) or morphological (e.g., *E. secundus*).

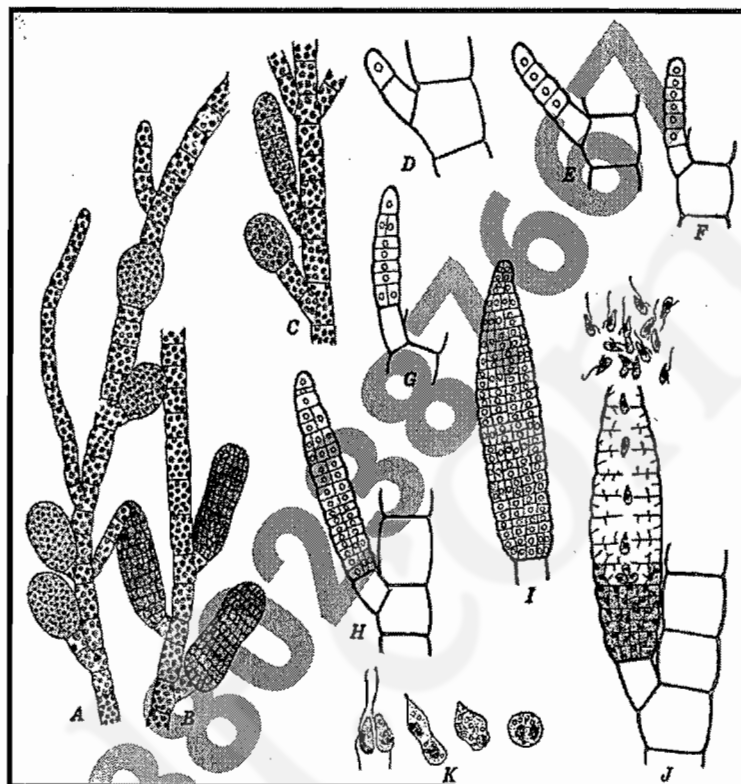


Figure 2: Spore formation in *Oedogonium*

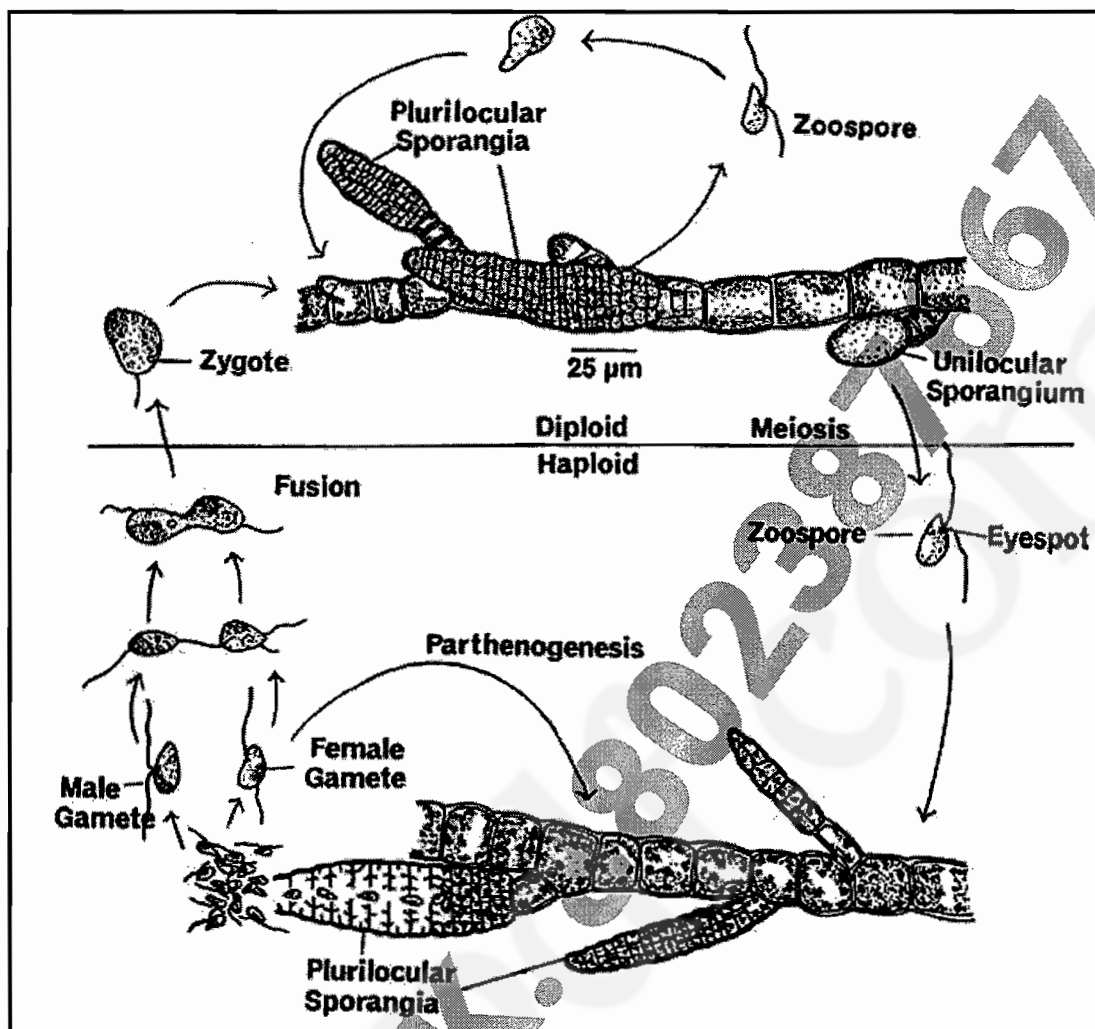


Figure 3: The life cycle of *E. siliculosus*

Gametes are formed in plurilocular gametangia which are similar to plurilocular sporangia in their structure and development. The uninucleate haploid protoplast, formed by meiosis, of each cell in plurilocular gametangia is transformed into a biflagellate pyriform gamete.

In *E. secundus*, which shows morphological anisogamy, two types of gametangia are produced. The megagametangia, with larger loci and larger gametes and the microgametangia, with smaller loci and smaller gametes. After their discharge from the gametangia, unequal gametes fuse in pairs to form zygospores.

In *E. siliculosus*, which shows physiological anisogamy, gametes come from different talli of different strains. The gametes of one strain are more active and behave as male gametes.

In most species, fertilization is facilitated by volatile sexual attractants, Sirenine & Ectocarpin (Butenyl cycloheptadiene). The male gametes, which may remain motile for about 8 hours, are attracted towards the female gamete by these substances. A large number of male gametes cluster around a female gamete (clump formation) and attach to it by their long anterior flagella. After sometime one of the male gametes contracts its flagellum and consequently comes in contact of the female gamete. These two gametes fuse to form zygospore.

The zygospore germinates within 2-3 days after fertilization. At the time of germination the diploid zygospore nucleus divides mitotically hence it gives rise to a diploid thallus. This thallus bears both unilocular and plurilocular sporangia.

Some unfused female gametes have the ability to germinate parthenogenetically and they form haploid filaments.

Vaucheria: An Account of Structure and Reproduction

About the Class Xanthophyceae

Vaucheria is a member of the class Xanthophyceae under the phylum Heterokontophyta with the following systematic position (R.E. Lee, 2008):

Division: Heterokontophyta

Class: Xanthophyceae

Order: Vaucheriales

Family: Vaucheriaceae

All the members of the class Xanthophyceae share the following features.

1. They are primarily freshwater and terrestrial algae with a few marine representatives.
2. The motile cells are with a forwardly directed tinsel flagellum and a posteriorly directed whiplash flagellum.
3. The chloroplasts contain chlorophylls *a* and *c*, lack fucoxanthin, and are colored yellowish-green.
4. The eyespot in motile cells is always in the chloroplast.
5. The chloroplasts are surrounded by two membranes of chloroplast endoplasmic reticulum. The outer membrane of the chloroplast E.R. is usually continuous with the outer membrane of the nucleus.
6. In most non-motile cells the wall is composed of two overlapping halves.

Molecular data have shown the Xanthophyceae is most closely related to the Phaeophyceae (Potter et al., 1997). Although the class is commonly called the Xanthophyceae, the proper name is the Tribophyceae since there is no genus in the class that can lend its name to Xanthophyceae (Hibberd, 1981).

About the Genus

There is only one genus, *Vaucheria* in the order Vaucheriales of Xanthophyceae.

Vaucheria has a relatively thin cell wall within which the cytoplasm is restricted to the periphery of the coenocyte, with the center being occupied by a large central vacuole (Fig. 1). In the cytoplasm, the numerous elliptical chloroplasts with pyrenoids are to the outside, whereas the nuclei are toward the center.

Growth of the filaments is restricted to the apex which has a large number of vesicles, mitochondria, and dictyosomes. Chloroplasts, nuclei, and the large central vacuole are not found at the growing tip. The large central vacuole contains lipids, degenerated chloroplasts, and crystals and extends the entire length of the filament except for the area immediately behind the growing tip. Cytoplasmic streaming takes place in the area of the large central vacuole and directly involves the nuclei, mitochondria, and their

associated dictyosomes. The cytoplasmic streaming involves two separate systems,

- a. the first based on microtubules that move the nuclei, and
- b. the second based on microfilaments that move the mitochondria and their associated dictyosomes.

The chloroplasts do not migrate regularly in patterns of definite streaming but *Vaucheria* is one of the algae that exhibit chloroplast orientation movements in the light.

Although *Vaucheria* can develop transverse septa that block off injured portions of the coenocyte, there is little reproduction by accidental breaking of filaments.

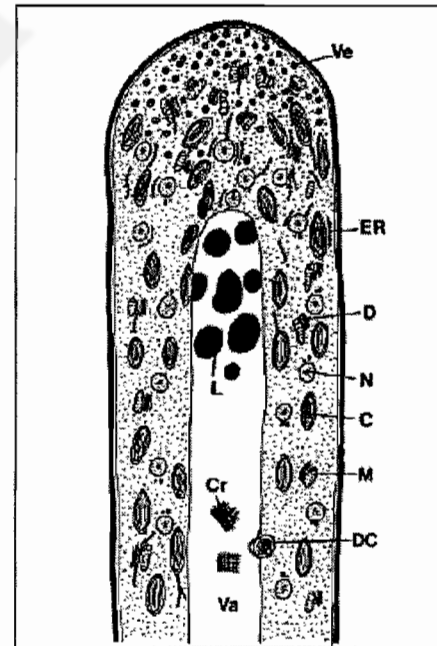


Figure 1: Schematic representation of the tip of a vegetative filament of *Vaucheria dillwynii*. (C) Chloroplast; (Cr) crystal; (D) dictyosome; (DC) degenerate chloroplast; (ER) endoplasmic reticulum; (L) lipid body; (M) mitochondrion; (N) nucleus; (Va) vacuole; (Ve) vesicle.

Reproduction

Vaucheria reproduces by both asexual and sexual means.

Asexual Reproduction

Asexual reproduction of aquatic individuals is usually by means of multi-flagellate, multinucleate zoospores, also known as **Synzoospores** (Fig. 2), which are produced singly in club-shaped sporangia at the swollen ends of filaments. In their production large numbers of chloroplasts and nuclei stream into the tip of the filament, the central vacuole decreases in size, and the tips appear dark green. A band of colorless protoplasm now appears at the base of the developing sporangium, due to which a septum is formed.

Within the sporangium, vesicles are produced (Ott and Brown, 1974b), around which nuclei become oriented with a pair of basal bodies between each nucleus and the vesicle membrane. Flagella are produced through the vesicle membrane, and the vesicles migrate to the plasmalemma. The nuclei with their flagella pairs thus come to lie in the peripheral area of the cell. The wall at the apex of the sporangium gelatinizes, forming a narrow aperture; the synzoospore pushes its way through the aperture and swims in the medium. The nuclei in the sporangium are separated from each other by a number of vacuoles, and one flagellum of each pair is slightly longer than the other (Greenwood et al., 1957). There is no eyespot or pyrenoid in the zoospore (Greenwood, 1959). The zoospores are sluggish in their movements, swimming for only about 15 minutes. On coming to rest, the flagella are withdrawn, and a thin wall is secreted. Germination follows almost immediately by the protrusion of one or two tubular outgrowths, one of which attaches itself to the substratum by means of a colourless lobed holdfast.

Instead of producing synzoospores, terrestrial individuals may have the entire contents of the sporangium develop into an **Aplanospore**. In such species too, synzoospores can be obtained if vegetative filaments kept moist for some days are soaked in water.

Akinetes are formed when terrestrial and aquatic species are subjected to very harsh conditions. The contents of the filaments divide into small segments by thick gelatinous walls. These thick walled multinucleate segments are called akinetes, cysts or hypnospores. The akinete protoplast is often laden with oil droplets. Cysts germinate under favourable conditions, each forming a new thallus. **Gongrosira stage** is a characteristic feature related to akinete formation by *Vaucheria* spp. Sometimes the protoplast of the cyst may divide into many uninucleate amoeboid segments that come out through a pore in the lateral wall of the cyst. At this stage, *Vaucheria* thallus resembles that of another genus, *Gongrosira*, and therefore, this stage is called as *Gongrosira* stage. Each amoeboid mass secretes a thin wall and eventually germinates to form a new thallus.

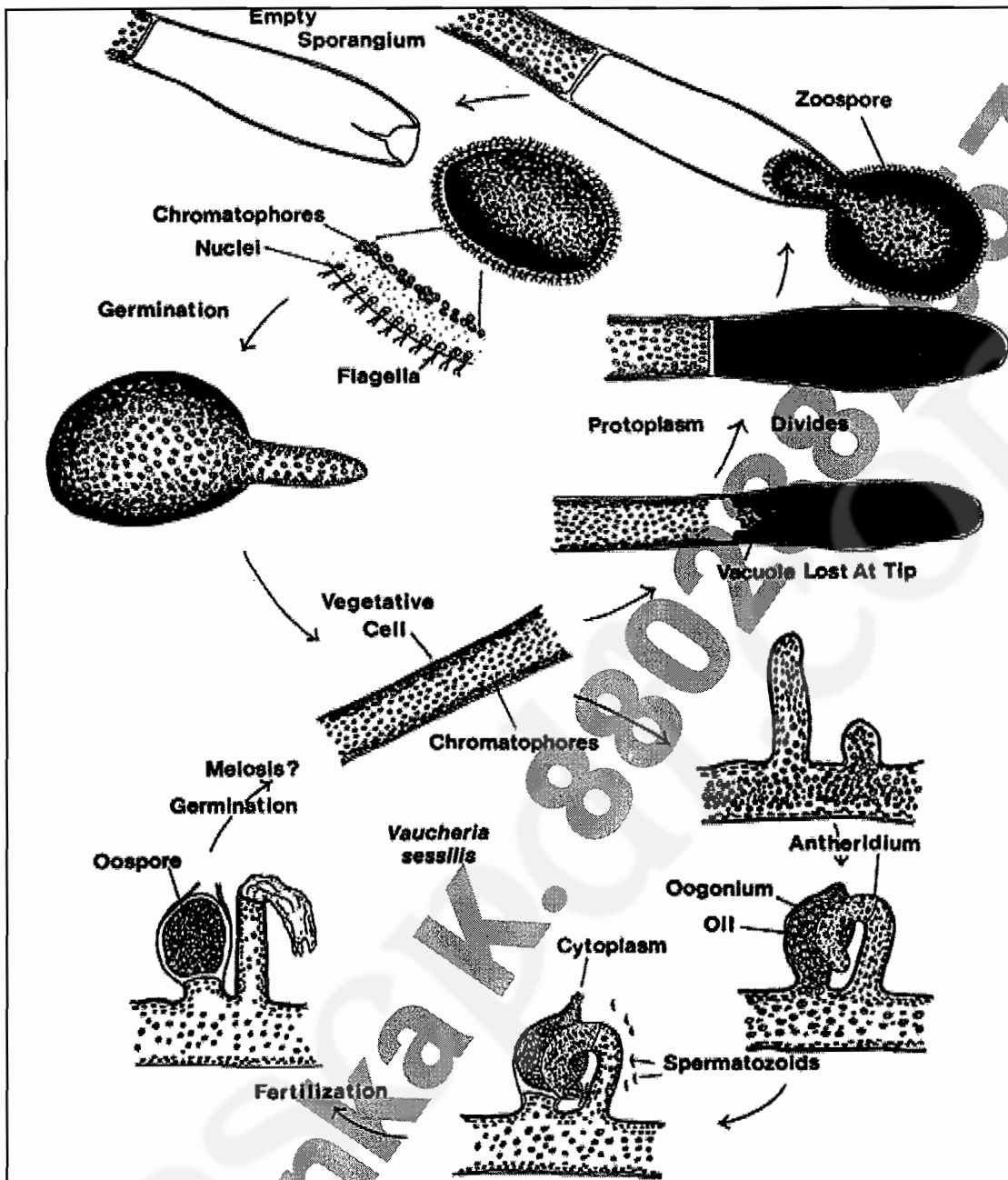


Figure 2: Life Cycle of *Vaucheria*

Sexual Reproduction

Sexual reproduction is oogamous and usually homothallic with meiosis occurring before the production of gametes (Al-Kubaisi and Schwantes, 1981). The life cycle is therefore **diplontic** with the diploid phase predominant.

Sex organs are common on filaments growing in damp soil or in quiet water, but are infrequent if they are growing in flowing water. The antheridia and oogonia are borne adjacent to each other and on a common lateral branch or on adjacent lateral branches. The sex organs are cut off by a septum.

The oogonium has a single egg and is filled with oil and chloroplasts. The mature oogonium produces a beak, the tip of which gelatinizes, forming an aperture. A portion of colorless cytoplasm of the egg projects through the aperture, and the egg contracts.

The antheridia usually develop as strongly curved cylindrical tubes that become cut off by a septum, usually fairly high up in the tube. The mature antheridium has the spermatozoids produced in a specific area between the central and peripheral cytoplasm. The central and peripheral cytoplasm contains those parts of the cytoplasm that are not included in the spermatozoids: the chloroplasts, vacuoles, and many

mitochondria (Ott and Brown, 1978). An aperture appears in the antheridium, and the spermatozoids are released. The spermatozoids are cylindrical posteriorly but have a flattened proboscis in the anterior portion (Fig. 19.9). There is a forward-projecting tinsel flagellum with two lateral rows of hairs, and a slightly longer trailing smooth flagellum. The nucleus is elongated and worm-like, as are the three or four mitochondria. There is neither a chloroplast nor an eyespot, but there is a Golgi body near the basal bodies of the flagella. The proboscis consists of eight or nine microtubules running beneath the plasmalemma with vesicles in between the microtubules.

Fertilization is accomplished by the spermatozoids fusing with the egg protoplasm through the aperture in the oogonium. The zygote secretes a wall, and the oil droplets fuse to form a small number of central droplets. The oospore is colored by the oil and the degeneration products of chlorophyll. It remains in the oogonium until it is liberated by the decay of the oogonial wall. The oospore then remains dormant for a few months before germinating.

Red Algae

General Introduction

The Rhodophyta (red algae) are a distinct eukaryotic lineage characterized by the accessory photosynthetic pigments phycoerythrin, phycocyanin and allophycocyanins arranged in phycobilisomes, and the absence of flagella and centrioles.

The Rhodophyta contains a single class: The Rhodophyceae. The Rhodophyceae are probably one of the oldest groups of eukaryotic algae. They most likely directly descended from the Glaucophyta.

This is a large assemblage of between 2500 and 6000 species in about 670 largely marine genera (Woelkerling 1990) that predominate along the coastal and continental shelf areas of tropical, temperate and cold-water regions.

Important features of the group include the following.

1. Primary photosynthetic pigment is **Chlorophyll a**. Chlorophyll d is also found in some species.
2. As accessory pigments, the Rhodophytes contain **phycobilins**, a class of water-soluble pigments. They are the only second group of eukaryotes to possess phycobilins apart from the Cryptomonads. Most important phycobilins possessed by the red algae are **R-phycoerythrin** and **R-phycocyanin**. These accessory pigments impart red, pink or violet colouration to the red algal thallus. Due to presence of phycobilins and lack of Chlorophyll b, it is concluded that the plastids of rhodophytes were gained independently from these other groups (Moreira, 2004).
3. Red algae are adapted to occupy deeper habitats, up to 879 ft (268 m), in the marine environment as they have the presence of the pigment R-phycoerythrin. This pigment absorbs blue light and blue light penetrates water to a greater depth than light of longer wavelengths. At great ocean depths, where the wavelength of light available for photosynthesis is very different from that in shallow water, the phycobilins become more active than the chlorophylls in absorbing light.
4. The rhodophytes show a unique ability of **Chromatic Adaptation**. In this, they can change the secondary pigment constitution of the body according to available light. This ability has also been called **Gaidukov Phenomenon**. It enables the rhodophytes to inhabit varying depths in the marine habitat.
5. With regards to thallus construction, some rhodophytes are unicellular but most species grow as filaments or membranous sheets of cells. Some of these multicellular forms are coralline, depositing skeletons composed of calcium carbonate crystals within and around their cell walls.
6. Rhodophytes also **lack the eukaryotic flagella** at any stage of the life history.
7. The cells of rhodophytes are commonly covered by a slimy outer sheath. Beneath the slimy sheath, there is a firm cellulosic wall. Additional colloidal compounds may be found in the cell wall, such compounds as **agars** and **carageenan**.
8. Rhodophycean cells may be multinucleate or polyploid.
9. The rhodophycean members have unstacked thylakoids in plastids. Moreover, there is no chloroplast endoplasmic reticulum (ER-Cp).
10. Asexual reproduction is generally present in most of the groups. It is based on non-flagellated spores such as **carpospores**, **tetraspores** etc.
11. Sexual reproduction is well developed and it is always **oogamous** type. The male gamete is a non-flagellated floating entity known as **spermatium** in most cases. The female gamete is known as **carpogonium**.
12. The life cycle of many red algae is extremely complex, involving one haploid phase and two diploid phases.
13. Rhodophytes store their energy surplus from photosynthesis in the form of **floridean starch**, a carbohydrate assembled from approximately 15 glucose units. This carbohydrate is unique to this group.

Importance of Rhodophytes

1. Some rhodophytes important in the formation of tropical reefs. These reef-building rhodophytes are called **coralline algae**, because they secrete a hard shell of carbonate around themselves, similar to the corals. This is an activity with which they have been involved for millions of years and in some Pacific atolls, red algae have contributed more to reef structure than corals.

2. Red algae are ecologically significant as primary producers, providers of structural habitat for other marine organisms.
3. Some red algae are economically important as providers of food and gels. For this reason, extensive farming and natural harvest of red algae occurs in numerous areas of the world. Two examples mentioned below are significant.
 - a) In Asia, rhodophytes are important sources of food, such as *Nori*. The high vitamin and protein content of this food makes it attractive, as does the relative simplicity of cultivation, which began in Japan more than 300 years ago.
 - b) Commercial *agar*, used as a culture medium for bacteria and other organisms as well as for other purposes, is produced from several genera of red algae. The so-called Irish moss is the source of *carrageenin*, a substance widely used as a stabilizing agent in emulsions and in ice cream.

Classification of Red Algae

The Rhodophyta has a single class, the Rhodophyceae.

In the past, the Rhodophyceae was divided into two sub classes,

1. the Bangiophycidae and
2. the Florideophycidae.

The Bangiophycidae were supposed to lack pit connections, apical growth, and probably sexual reproduction, where as the Florideophycidae had pit connections, apical growth, and sexual reproduction with a triphasic life cycle.

The Bangiophycidae have since been found to have pit connections and apical growth in the Conchocelis filamentous stage of the Bangiaceae. Sexual reproduction also occurs in the Bangiaceae. In turn, the Florideophycidae do not necessarily have apical growth (intercalary growth occurs in the Corallinales), nor do they all have a triphasic life history (e.g., red algae in the Batrachospermales). For the above reasons, the two subclasses have been dropped, as suggested by Gabrielson (Gabrielson, et al., 1985).

The classification at the level of orders of the red algae is based on complex characteristics of sexual reproduction.

RE Lee (2008) has identified the following ten orders of Rhodophyceae.

1. Order Cyanidiales: unicells that inhabit volcanic areas with pH values ranging from 0.5 to 3.
2. Order Porphyridiales: unicells, or multicellular algae that are held together by mucilage.
3. Order Bangiales: plants having a filamentous phase with pit connections and a macroscopic phase without pit connections.
4. Order Acrochaetales: algae with a uniseriate filamentous gametophyte and tetrasporophyte (if both are present).
5. Order Batrachospermales: uniaxial (one apical cell per branch); gonimoblast usually develops from the carpogonium or hypogenous cell.
6. Order Nemaliales: multi axial (more than one apical cell per branch); usually the gonimoblast develops from the carpogonium or the hypogenous cell.
7. Order Corallinales: heavily calcified algae with the reproductive organs in conceptacles.
8. Order Gelidiales: fleshy agarophytes, carpogonial branch consisting of a single cell, the carpogonium, no differentiated auxiliary cells.
9. Order Gracilariales: fleshy agarophytes, two-celled carpogonial branch, no auxiliary cells, or connecting cells.
10. Order Ceramiales: relatively delicate or filamentous forms with an auxiliary cell cut off after fertilization and borne on the supporting cell of a four-celled carpogonial filament.

Polysiphonia life cycle

An introduction

Polysiphonia is a red alga, with the following systematic position.

Phylum: Rhodophyta

Class: Rhodophyceae

Order: Ceramiales

The members of this order of the red algae have the auxiliary cell cut off after fertilization and borne on the supporting cell of the four-celled carpogonial filament. Most of these algae are relatively delicate filamentous or membranous forms.

Ecology

Polysiphonia is a large genus with about 200 species. The genus is represented in India by about 16 species found in southern and western coasts of India. Some common Indian species are *P. ferulacea*, *P. urceolata* and *P. variegata*.

Polysiphonia species occur either as lithophytes or as epiphytes on other algae.

Thallus structure

The thallus is filamentous, red or purple red in colour. The thallus is multi-axial and all cells are connected by pit connections hence, the name given is *Polysiphonia*. Due to continuous branching and re-branching the thallus has feathery appearance (Fig. 1A). The thalli may reach the length of about 30 cm.

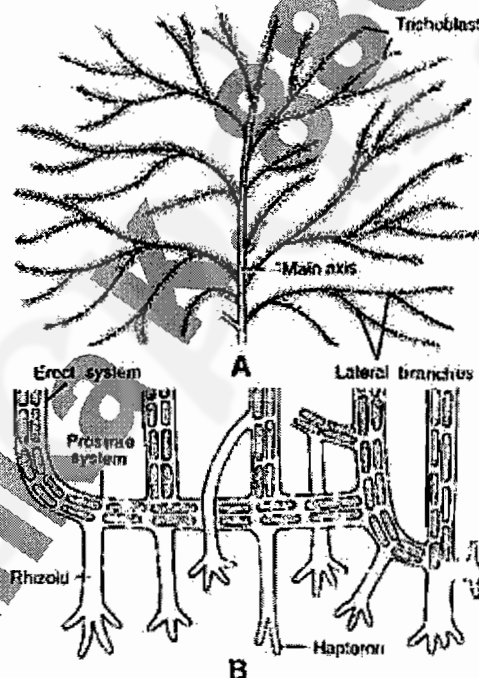


Fig. 1 (A, B). *Polysiphonia*. External features. (A) Habit, (B) Prestrate and erect system

When the species grows on a solid substrate, some of the polysiphonous axes creep over the substratum to which they are firmly anchored by thick-walled, lobed rhizoids (Fig. 1 B). When it grows as an epiphyte on another alga, the rhizoids penetrate that host tissue.

In *Polysiphonia* the uninucleate, dome-shaped apical cell is polyploid and contains 64 to 128 times the amount of DNA in most of the mature cells in the alga (Goff and Coleman, 1986). Division of the cells derived from the apical cell is usually not accompanied by DNA replication; therefore, the farther the daughter cell is from the apical cell, the lower the ploidy of the cell, until the ploidy number stabilizes at 1n.

The thallus is made of a central large filament or central siphon of cylindrical cells. The central siphon is surrounded by a number of pericentral cells or pericentral siphons (Fig. 2 A, B, C). The number of pericentral siphons varies from species to species.

Each pericentral siphon remains connected with central siphons through pit connections. The successive central siphon cells and all peripheral cells are also connected to each other through pit connections. Hence the complete thallus makes a polysiphonaceous structure (Fig. 2 C).

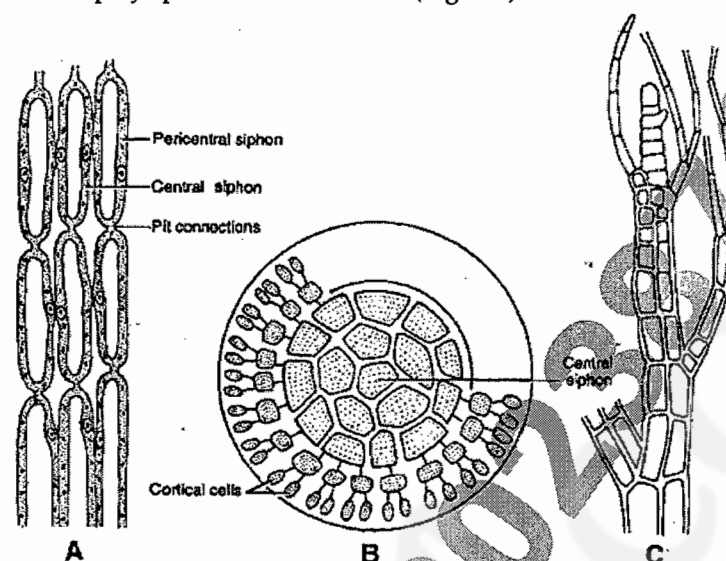


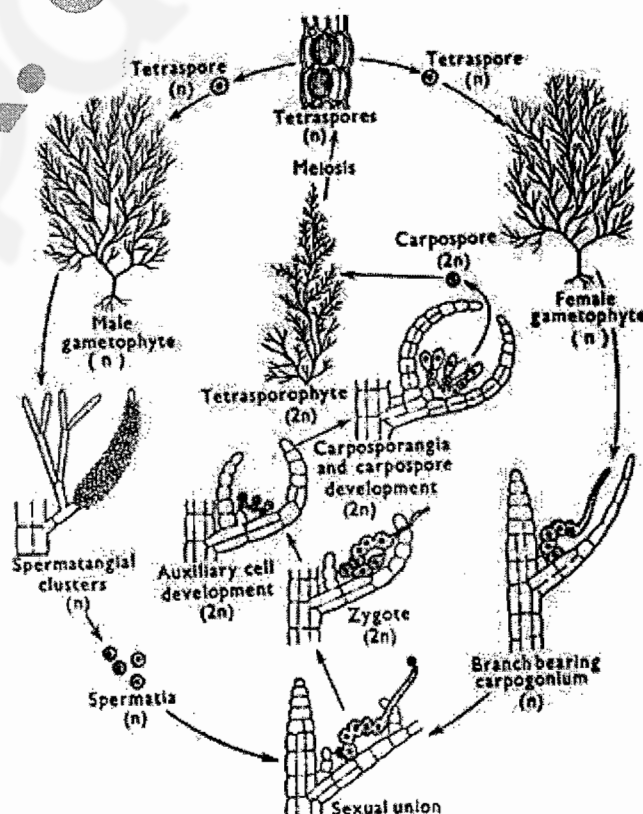
Fig. 2 (A-C). *Polysiphonia*. Thallus structure. (A) Part of aerial branch, (B) T.S. of aerial axis, (C) Vertical section of main axis.

The apical cell forms daughter cells that produce lateral branches before dividing longitudinally into central and pericentral cells. The pericentrals are the same length as the axial cells. The lateral branches are of two kinds: the ordinary branches and the trichoblasts. The ordinary branches are polysiphonous with unlimited growth, similar to the main axis. The trichoblasts are uniseriate, usually colorless, and bear the sex organs.

Life cycle

Polysiphonia is heterothallic and shows a triphasic life cycle. In the life cycle of *Polysiphonia* three kinds of thalli are found. These are:

- The gametophytic thalli which are haploid free living and dioecious. The male sex organs spermatangia are formed on male gametophytic plant and the female sex organs carpogonia are formed on female gametophytic plant.
- The carposporophytes are diploid, depend upon the female gametophyte. They develop after fertilization from zygote and later bear carposporangia. The carposporangia form diploid carpospores.
- The tetrasporophytic plant which is formed by germination of diploid carpospores is diploid and independent. Then plant bears tetrasporangia which form four haploid tetraspores which again give rise to male and female gametophytic plants.



In life cycle of *Polysiphonia* both asexual and sexual reproduction takes place. The life cycle is example of triphasic alternation of generation.

Algal Habitats & Their Distribution in India

Tiffany (1951) while discussing fresh water ecology of Algae recognized six major categories of algal habitats as follows:

I. Hydrophytes (Aquatic algae)

1. Benthophytes (growing on mud and other bottom)..
2. Epactiphytes (growing along shores of lakes or ponds).
3. Thermophytes (growing in thermal waters).
4. Planktophytes (floating algae).
 - (i) Euplanktophytes (true floating algae).
 - (ii) Tychophytes (become floating due to accident).
5. Halophytes (growing in salt lake or so).
6. Epiphytes (growing attached on other plants).
7. Epizoophytes (growing attached on animals).

II. Edaphophytes (soil algae)

1. Saphophytes (surface algae, entirely exposed).
2. Cryptophytes (subterranean, not generally exposed).

III. Aerophytes (air algae)

1. Epiphilophytes (growing on leaves).
2. Epiphloeophytes (growing on barks).
3. Epizoophytes (growing on animals).
4. Lithophytes (growing on or in rocks).

IV. Cryophytes (snow algae)

V. Endophytes (growing within other plants).

VI. Endozoophytes (growing within animals).

Strictly not following this pattern and including marine algae more, outline details of different categories are discussed here as follows:

Planktonic Algae

The term planktonic alga refers to the forms found floating or weakly swimming in water. These forms may be uniformly distributed in water or may be discontinuous and patchy, both horizontally and vertically. These may form a layer just 1 cm thick and from 1 to 10 m broad. In ocean, elliptical patches may be 60 to 240 km in size. In fresh water reservoirs, during winter and spring, diatoms and during late spring, summer and autumn blue green algal forms predominate while in sea and oceans, dinoflagellates during summer and diatoms in rest of the seasons predominate. Among the fresh water planktonic algae, forms such as *Chlorella*, *Scenedesmus*, *Hydrodictyon*, *Chlamydomonas*, *Volvox* and *Eudorina* of Chlorophyceae; *Euglena* and *Phacus* of Eugleninae; *Microcystis*, *Anabaena*, *Aphanothece*, *Spirulina*, *Arthrospira*, *Anabacnopsis* and *Oscillatoria* of Myxophyceae and *Melosira*, *Cyclotella*, *Pinnularia*, *Navicula*, *Fragilaria* and *Asterionella* of Bacillariophyceae are common while among marine planktonic forms *Phaeocystis*, *Dinophysis*, *Exuviaella* and *Prorocentrum* of Desmophyceae; *Gymnodinium*, *Peridinium*, *Gonyaulax* and *Ceratium* of Dinophyceae; *Skeletonema*, *Cyclotella*, *Planktoniella*, *Eucampia*, *Hemidiscus*, *Chaetoceros*, *Biddulphia*, *Fragilaria*, *Asterionella* and *Nitzschia* of Bacillariophyceae; *Trichodesmium*, *Anabaena*, *Oscillatoria* and *Aphanothece* of Myxophyceae and *Chlamydomonas* of Chlorophyceae are well-known.

Quite frequently abundant growth of planktonic algae begins to impart colour to the water. Such a phenomenon is called water bloom and as caused by algae may be called algal bloom. Formation of algal blooms fairly depends upon factors like temperature increase, longer days and nutrient availability. These

water blooms may be temporary or permanent and mixed or pure. Temporary and mixed types of water bloom are produced by simultaneous growth of *Chlamydomonas*, *Scenedesmus*, *Chlorella*, *Ankistrodesmus*, *Pediastrum* etc. While temporary and pure (containing only one algal species) water bloom produced often by *Volvox globator* or *Chlamydomonas* spp. are quite common in inland waters during rains and winters. Blue green algae by far play a vital role in bloom formation. During late winters and summers blooms containing *Anabaena*, *Anabaenopsis* and *Microcystis* are seen at various places in tropics while *Trichodesmium* forms a permanent red water bloom in red sea (this name of the sea is probably after it) and *Microcystis aeruginosa* forms permanent blue green water bloom in temple ponds and other permanent water reservoirs in India. Water blooms often produce foul odour and make water unpotable. *Microcystis* and *Gymnodinium* like algae secrete toxic or poisonous substances in water (see Phonemic Importance of Algae).

Benthic Algae

The term benthic algae refers to aquatic algae found attached to one or the other substratum. Among the fresh water forms, *Cladophora*, *Pithophora*, *Chara*, *Nitella* etc. and among marine forms most members of Phaeophyceae and Rhoeophyceae are the common examples. In modern terminology, term *periphyton* is used to algae found attached to variety of substrates. Term *epi-phyte* applies to plants attached to other plants. *Epipellic* algae are those found growing on and in sediments, *epipsammic* algae are located on or in sand and *epiithic* algae are located on rocks.

Some benthic forms are annuals such as *Cladophora*, *Enteromorpha*, *Porphyra* and *Polysiphonia* while quite many of them are perennials such as *Fucus*, *Scragassum*, *Laminaria*, *Chonarus*, *Gracilaria*, *Acetabularia* etc. Among marine benthic forms *Ulva*, *Ectocarpus*, *Sphacelaria* *Acetabularia* are cosmopolitan but most marine forms have restricted distribution. The prolific growths of benthic forms are found on rocky coasts such as in the areas of Rameshwaram in South India and Dwarka, Okha etc. in West India. The sublittoral zones of Indian Ocean by far offer the highest net primary productivity by algae in the world (up to 2000 g C m⁻² yr⁻¹). Large benthic marine algae are economically very important in various ways to our civilisation

Thermal Algae

Thermal algae have been a subject of considerable interest for many years. Some algae withstand or tolerate a very high temperature and these are often called thermal algae. Such forms are known to grow up to 85°C, nearly boiling water. There is no adequate explanation for their ability to survive at such a higher temperature. Evidently their proteins and the mechanism for making proteins are more resistant to high temperature (in general, protein denatures above 50°C). Several explanations have been proposed to account for the growth of such algae which include peculiar organisation of their protoplasm, their primitive morphology, absence of certain structures such as mitochondria, etc.

Based on temperature, algae have been classified: *Hypothermae* (below 18°C), *Hliarothermae* (18—30°C), *Euthermiae* (30—50°C), *Acrothermae* (50—70°C), *Hyperthermae* (above 70°C).

Majority of thermal algae belong to Myxophyceae, e.g. *Synechococcus elongatus*, *Mastigocladus laminosus*, *Phormidium tenue*, *Conferva thermalis* etc. A few forms belong to Chlorophyceae (Zygnematales) and Bacillariophyceae. Thermal algae reproduce by means of cell division and fragmentation and very rarely by spores.

Considering the ability of these organisms to withstand in extreme adverse conditions, we proposed Relict hypothesis. According to which occurrence of such forms shows the possible existence of this form of life during the early history of earth when it was supposed to have been covered with hot and highly mineralised water. Such plants thus represent the earliest link in the chain of evolution.

Soil Algae

Such forms of algae that grow on or in soil are called soil or terrestrial algae or edaphophytes. Term *edaphon*, a counterpart of plankton, was proposed by France (1913) to include soil algae. It is now long known that a definite algal flora grows on soil. *Vaucheria*, *Botrydium*, *Zygnema*, *Cedogonium*, *Microcoleus*, *Nostoc*, *Oscillatoria* etc. occur on soils. Algae are found both on the surface of the soil (*saphophytes*) and at depths of a few inches to a few feet (*cryptophytes*). Friedmann and Ocampo (1976) while studying desert soil algae recognised different categories as—*endedaphic* (living in soil), *epidaphic* (living on the soil surface), *hypolithic* (on the lower surface of stones on soil). It is thought that the algae growing beneath the surface have been washed there during rains, abetted perhaps by earthworm tunnels and cultivation. Since these live in darkness, these must survive as saprophytes.

The biological significance of soil algae is great. Higher plants like Bryophytes are believed to have evolved from them. This seems a natural conclusion based on the abundance of algae and the similarity of physiological processes to those of higher plants. Certain blue green algae are capable to fix atmospheric

nitrogen (see topic Role of Algae in Nitrogen fixation). *Nostoc*, *Anabaena*, *Scytonema* and *Tolypothrix* are well known for their ability to fix atmospheric nitrogen and thereby increasing soil fertility.

Cryophytes

Certain algae are found growing on snow covered peaks of high mountains imparting attractive colours to snow. Common examples are—*Haematococcus nivalis* (imparting red colour to arctic and alpine regions), *Chlamydomonas yellowstonensis* (yellow green colour to snow in yellow stone National Park of America), *Raphidonema* (imparting green colour to European mountains), *C. lindrocysiis*, *Protoderma*, *Scotiella* etc. *Ancylonema nordenskioldii* imparts brownish to purple colour to snow.

Lithophytes

The algae growing attached to stones and rocky surfaces are called lithophytes. These may be of two types.

(i) *Epilithic*. These include algae living on surface of rocks, e.g. *Caiothrix*, *Rivularia*, *Gloeocapsa*, *Plcurocapsa*, *Ectocarpus*, *Polysiphonia* etc.

(ii) *Endolithic*. These include algae which live inside the rocks, e.g. *Dalmatella* and *Pudocapsa*.

Epiphytes

Some algae grow attached on the other plants and are called epiphytes. Such algae do not obtain the food from the plants on which they grow rather require support only. A few species of *Bulbochaete*, *Oedogonium*, and *Ulothrix* etc. grow on other larger algae. Besides, *Coleochaete* in association with *Chara* and *Nitella*, *Chaetophora* on leaves of *Vallisneria* and *Nelumbo* and *Oedogonium* on *Hydrilla* are seen frequently growing in nature as epiphytes. The algae that grow on the surface of leaf are called epiphylliphytes, e.g. *Chaetophora* and *Cornospogan* and others that grow on the surface of bark are epiphloeophytes, e.g. *Scytonema*, *Lyngbya*, *Aphanocapsa*. Tree bark inhabiting algae are also called *Corticolous*.

Halophytes

Certain algae inhabit in water with high percentage of salts such as *Dunaliella* and *Stephanophora*. However, *Chlamydomonas ehrenbergii* and *Ulothrix flacca* have also been reported to grow in such water.

Symbionts

A pretty large number of algae live in association with dissimilar organisms for their mutual advantage and are called symbiotic algae. The common examples of such association are the presence of *Nostoc* in *Anthoceros*, *Anabaena cycadae* in the coralloid root of *Cycas*, *Anabaena* species in *Azolla* etc.

However, lichens are the best examples of symbiosis where the association lies in between algae and fungi. Some thirty genera are known to behave as phycobionts (algal components of lichens), e.g. *Trebauxia*, *Caiothrix*, *Chlorella*, *Gloeocapsa*, *Nostoc* etc. The example of symbiosis between algae and animals is the occurrence of *Cladophora* on snails.

Endozoic Algae

Endozoic algae inhabit the protoplasm of other organisms, e.g. *Euglenomorpha*, *Zoochlorellae*, *Zooxanthellae*, *Carteria* etc. *Euglenomorpha* is found in the intestine of tadpole of *Rana* (frog) whereas, *Zoochlorellae* occur in the coelome of *Hydra* and several other invertebrates. *Chlorella*—like algae are found living within *Paramecium*, *Hydra* and certain molluscs and sponges (Cooke, 1975). *Zooxanthellae* live in intimate association with coral community. The photosynthetic activity of the algae is of primary importance to these organisms. Trench (1977) has demonstrated that ^{14}C labelled products of algae rapidly appear in the lipids and proteins of host animals. To maintain this relationship, some animals develop unusual habit, e.g. Pearse (1974) found that sea anemones containing algae were phototactic while those lacking them did not move to or from light of varying intensity. The association between green alga *Platymonas* and flatworm *Convoluta roscoffensis* reveals interesting fact. Flatworms are dependent for their development on the presence of alga within it. Alga probably releases amino acids to the animal.

Parasitic Algae

Some algae, for their food, are dependent on other plants and are termed as parasitic forms. The common intercellular parasite *Cephaleuros* (Chlorophyceae) grows on the leaves of angiosperms like *Magnolia*, *Rhododendron*, *Thea sinensis*, *Ficus*, *Mimusops*, *Hexandra*, *Psidium gujava* etc. The disease caused by *Cephaleuros* on Tea Plants (*Thea sinensis*) is popularly referred as "Red rust disease of Tea." Another alga *Phyllosiphon* (a member of Chlorophyceae) occurs as a parasite on an angiosperm *Arisarum vulgare* (Araceae).

Polysiphonia fastigata is a semiparasite occurring on the another algae *Ascophyllum nodosum* (Phaeophyceae). Some blue green algae *Anabaenium*, *Oscillatoria* and *Simonosilla* are found as parasite on man and in the intestines of animals. *Chlorochytrium lemnae* lives as a space parasite inside the tissue of an angiosperm *Lemna*, *Phyllobium sphagnicolum* grows as a true parasite on the leaves of a bryophyte *Sphagnum*.

Distribution of Marine Algae in India

A greater part of 3850 miles long Indian coastal line, except only in some restricted areas such as lagoons, lakes and deltaic formations of large rivers like Ganges, Mahanadi, Godavari, Krishna, Cauvery etc., is regular and without indentation. Some places of interest where the collections of algae have been made from time to time are—Okha, Dwarka, Muldwarka, Bombay, Karwar, Travancore, Cape Comorin, Rameshwaram, Pamban, Krusadai, Shingle, Hare Island, Church Island, Manar, Mahabalipuram of Seven Pagodas near Madras etc. Besides, Andamans, Nicobars, Laccadives and Minicoy are also important islands included in Indian sea territory. Growth of marine algae in certain restricted areas is considerably influenced by the environmental factors such as the nature of substratum, tide, surfaction, clearness of water etc.

On record, Indian marine algal flora is largely represented by Chlorophyceae (34 genera and 100 species), Phaeophyceae (32 genera and 93 species) and Rhodophyceae (104 genera and 253 species). An appreciable amount of work on distribution of marine algae has been done by J.N. Misra. The work on marine Myxophyceae has been compiled up by Desikachary who states that some 50 species are marine. However, data of marine algae is still not complete and needs more exploration.

The distribution of Indian marine algae can be safely studied by dividing marine coasts into two parts.

Algae of East Coast of India

In the East Coast, in Nellore district of Tamil Nadu, there is a shallow water lake. Pulicat lake, extending upto 37 miles in length and 3-11 miles in breadth. It is separated from the open sea by a narrow sandy island but is communicated to later in the north of Pulicat. Yet another lake called Chilka lake, which is slightly larger than Pulicat lake, is further up in the north. This lake is separated from the open sea by a narrow sandy ridge about 200 yards wide). While it is nearly 44 miles long and 5 to 20 miles wide, it is connected with the Bay of Bengal by a single narrow mouth. During summer months the lake contains predominantly saltish water but during rainy season because of discharge of two rivers, Bhargavi and Daya, it becomes almost a fresh water lake. Sundari bans, the remaining part of East Coast, which forms the lower part of Ganges Delta, runs about 170 miles long along the sea face of Bengal. The entire area is a tangled network of streams, rivers, creeks and inlets enclosing large number of islands of various shapes. In this region, Andamans and Nicobars are also considered for studying marine algae distribution in India.

Chlorophyceae- Chlorophyceae in East Coast of India is represented by 18 genera and 38 species. *Ulva*, *Cladophora*, *Bryopsis*, *Acetabularia*, *Neomeris*, *Udotea*, *Dictyosphaeria*, *Boodleia*, *Halimeda* and *Caulerpa* are the important genera of the area. A few species such as *Chaetomorpha littorea*, *Rhizoclonium kernerii*, *Caulerpa fergusonii*, *C. freycinetii* etc. are restricted to only East Coast region of India.

Phaeophyceae- So far 21 genera and 41 species of Phaeophyceae have been reported from this region. These belong to Ectocarpales, Sphacelariales, Dictyotales and Fucales. The order Ectocarpales, represented by 9 genera and 18 species, is the dominant group constituting about 50 per cent of the total Phaeophyceae of this region, it is represented by *Ectocarpus*, *Giffordia*, *Streblonema*, *Hecatonema*, *Chnoospora*, *Colpomenia*, *Hydroclathrus*, *Ivengaria* and *Rosenvingea*. The genus *Chnoospora* is not found elsewhere but for this region. The two species of *Sphacelaria*, *S. tribuloides* and *S. Jurgigera*, are universally occurring. The order Dictyotales is fairly represented by majority of its genera, i.e. *Dictyota*, *Dictyopteris*, *Padina*, *Spatoglossum*, *Stoechospermum*, *Zonaria* and *Procockiella*.

However, order Fucales is poorly represented. Only four genera, *Hormophysa*, *Cystophyllum*, *Sargassum* and *Terbenaria* are found. Of these *Turbenaria* is localised to southern part of both the coasts (East as well as West Coasts of India).

The common species of various forms are—*Ectocarpus brevarticulatus*, *E. Jilifer*, *E. enhali*, *E. geminifructus*, *Chnoospora minima*, *Sphacelaria tribuloides*, *S. furcigera*, *Dictyota dioxotoma*, *Padina gymnospora*, *P. tetrastromatica*, *Turbenaria conoides*, *Zonaria latissima*, *Z. crenata*, *Dictyopteris delicatula*, *D. muelleri*, *Sargassum wightii*, *S. cristaeifolium* etc.

Rhodophyceae- Forty-nine genera and ninety two species represent the rhodophycean flora of East Coast of India. Of these forty three species are known so far from this region only. A few of them are *Acrochaetium iyengarii*, *Sciania bengalica*, *Chondria armata*, *C. transversalis*, *Acanthophora muscoides*, *Polysiphonia unguiformis*, *P. tuticorinensis*, *Rhodymenia dissecta*, *Liagora erecta*, *Porphyra tenera*, *Martensia fragillilis*, *Gracilaria disticha*, *G. lichenoides*, *Ceramium gracillimum* etc.

Algae of West Coast of India

Of the West Coast, the greater part is continuous and without indentation. There are two important gulfs in this region: (i) the Gulf of Cutch and (ii) the Gulf of Cambay. The Gulf of Cutch is in North-West of Saurashtra and is an inlet of West Coast of India. The port Okha is situated at its entry. The Gulf of Cambay is a narrow strip along the Arabian Sea. It separates Saurashtra from Northern Coast of Bombay. About Bombay, back waters or lagoons are common which have been transformed by the gradual sinking of coastal areas in the past. From Bombay to South Malabar, the coast is continuous. The coast is again interrupted by inlets and back waters along the Cochin and Travancore coasts. In this area, the large mud banks, locally called 'Kayals' are quite famous. Besides, when studied the marine algal flora of India, the Laccadives and Minicoy are also taken into Account.

Chlorophyceae- West coast algal flora is enriched with 28 genera and 72 species of Chlorophyceae. A few characteristic species are *Enteromorpha tubulosa*, *Ulva reticulata*, *Bryopsis hynoides*, *Acetabularia moebii*, *Struvea anastomosans*. *Caulerpa* with its several species is quite common throughout the coast.

Phaeophyceae- From West Coast of India 28 genera and 70 species have been recorded. All the four orders common to East Coast have also been recorded from this region. Order Ectocarpales, represented by 17 genera and 31 species, is again the dominant group that constitutes nearly 50 per cent of the Phaeophyceae found in this region. *Giffordia mitchellae* is widespread throughout the region. *Tyngaria* is particularly common in the littoral regions of Dwarka and Okha. Certain genera such as *Liebmannia*, *Myrionema*, *Levringia* and *Nemacystius* are categorically restricted to this region. *Dictyota* is quite common to both coasts and is commonly represented by *D. dichotoma*, *D. barta-yresiana*, *D. dumosa* and *D. maxima*. Fucales are again poorly represented. Only five genera are known from this Coast. Besides all the four common to East Coast, *Cystoseira* is the fifth one. Genus *Sargassum* is richest both in its species and luxuriance of growth and is comparatively greatly developed than that of East Coast. Its three species are common to East Coast also (*S. wightii*, *S. cristaefolium* and *S. myricocystum*). The common forms are *Ectocarpus arabicus*, *E. enhali*, *Giffordia mitchellae*, *Colpomenia sinuosa*, *Lyngaria sfellata*, *Rosen-vingea intricata*, *Sphacelaria tribuloides*, *S. furcigera*, *Dictyota dichotoma*, *D. divaricata*, *D. cervicornis*, *Dictyopteris australis*, *Padina gymnospora*, *P. tetrastrum*, *Spatoglossum variable*, *Cystophyllum muricatum*, *Sargassum tenerrimum*, *S. cinereum*, *Turbenaria decurrens* etc.

Rhodophyceae- West Coast is quite rich as regards Rhodophyceae being represented by 89 genera and 175 species. A few most characteristic forms are *Scinaia hatel*, *Asparagopsis taxiformis*, *Nitophyllum punctatum*, *Rhodymenia australis*, *Hypoglossum spathulatum* etc.

Boergessen, Anand and others have studied marine algal flora in details and found that 12, 7 and 49 species belonging to Chlorophyceae, Phaeophyceae and Rhodophyceae are endemic to India. Of these 68 species, nineteen are restricted to East Coast and forty-six to West Coast and three are common. It is important to note that both *Laminaria* and *Fucus* are not found in Indian marine water (Misra, 1956).

Sexuality of Algae

Origin of sex- The presence of sexual stages in certain algae is an advanced feature over very primitive forms such as bacteria, virus and blue green algae. Blue green algae, a class of Algae, reproduce either vegetatively or asexually. Despite the approaches made during last twenty years about the existence of some degree of sexual recombination (genetic recombination, transformation and transduction), no concrete evidence of sexual reproduction could be established in this class.

The question on origin of sex is debatable and without definite preponderance. However, a propounded theory called "starvation theory" or "hunger theory of sex" reveals that origin of sexuality is the result of an attraction between nutrition depleted cells. Such cells fail to develop unless union (act of sexual reproduction) occurs.

In sexually reproducing algal forms, the asexual reproduction is not completely replaced by the sexual methods. Thus sexual reproduction appears to be a supplementary method of multiplication. In certain cases such as in *Ulothrix* and *Chlamydomonas* since gametes and zoospores are more or less similar in structure, it is believed that such gametes might have originated or derived from zoospores. Comparatively, the gametes are produced in larger number but are smaller in size. Quantitatively they have reduced amount of protoplast as compared to zoospores. It may be one reason due to which gametes becomes unable to develop into a new plant directly as produced by zoospores. The gametes then may be called as smaller zoospores. Without denying the fact that the mode of formation of zoospores and gametes are similar, how the mere variation in size can account a fundamental difference in their behaviour, is a question worth to be emphasised.

In lower organisms, the sexual reproduction occurs during unfavourable conditions and so long conditions are favourable, the asexual reproduction continues. Then it will not be improper to confess that in an enfeebled condition caused by starvation, smaller zoospores incapable of forming new plants are produced. Owing to their incapability, they pair, combine their resources and form zygote to regain the power of producing a new individual. The thick-wall-ed nature of zygotes enables them to tide over an unfavourable period for growth, constitutes a further support to the theory. However, in certain cases, the recorded change of behaviour such as zoospores into gametes and vice versa, provides additional support to the same concept. Some examples discussed under relative sexuality also support this view point.

The above discussion some how admonishes the hunger theory of sex. However, several experiments have been carried out to establish the fact but there remains a lot to substantiate this concept. Even then the starvation theory is quite appealing, and it has been widely accepted.

Relative sexuality- Hartman (1923) propounded the hypothesis that all gametes contain both male and female potentialities. It is predominating potentiality which decides male or femaleness of the gametes. While working on determination of relative sexuality of male gametes of *Chlamydomonas*, Moewus (1939) recognised the following four categories:

- (i) No fusion of gametes,
- (ii) Isolated pairs of fusing gametes,
- (iii) Clumps of ten to twenty gametes, and
- (iv) Clumps of hundred or more gametes.

Of these, the last three categories are explained on the basis of different intensities of maleness among male gametes and femaleness among female gametes. Similarly several examples of relative sexuality in *Dasycladus*, *Poly-loma*, *Enteromorpha* and *Protosiphon* are also known. Such examples support Hartmann's hypothesis. Hartman further reported that in *Ectocarpus siliculosus* occur some unusual gametes. These were able to unite both with gametes of opposite sex and with the gametes of their own sex. He coined the word 'relative sexuality' for such varying observations.

Evolution of sex- Of three kinds of sexual reproduction—*isogamy*, *anisogamy* and *oogamy*, *isogamy* is the most primitive which marks the transition from asexual reproduction to sexual reproduction. Isogametes are similar to zoospores but smaller in size. In isogamous reproduction, both kinds of gametes are morphologically similar in size and shape and are motile. Isogamy occurs in several green and brown algae such as *Chlamydomonas debaryanum*, *Ulothrix*, *Cladophora*, *Ectocarpus* etc. and is accomplished by the fusion of two similar, motile and naked gametes. A marked physiological difference between them due to dissimilar behaviour is observed in certain cases. In *Ulothrix zonata* isogametes fuse only if they are produced in distinct filament. Such forms with functional disparity between isogametes are a step ahead in evolution of

sex and such gametes are called + (plus) and—(minus) strains due to lack of male and female characteristics.

Anisogamy is advanced over isogamy and is witnessed in *Chlamydomonas braunii* and *Pandorina* spp. Here the female gametes are larger in size than male ones but both kinds of gametes are motile.

Still more evolved sexuality is oogamy where male reproductive organs are called antheridia and the female oogonia. The gametes are recognisable as male (sperm or antherozoids) and female (egg or ovum). During oogamy, the female reproductive organ increases in size to store more food for the unfavourable periods while male sex organ gives rise to innumerable small gametes just to ensure fertilisation. Oogamy is known in *Chlamydomonas coccifera*, *Eudorina* etc. but distinct development of antheridia and oogonia are met in *Oedogonium* and *Vaucheria* where eggs are not only non-motile and stationary but, fertilisation also takes place in situ i.e. in the oogonium. *Chara* is still advanced where the elaboration and differentiation of sex-organ reaches its culmination point in the green algae. In *Chara*, both the sex organs remain enclosed by a sterile jacket layer of cells. The male reproductive bodies are called globule and female nucule. Vaidya (1970) have proposed the name Spermatogamma and oogama to male and female reproductive bodies due to their complex structures. *Chorales* are unparallel in this regard as no such condition is observed in any other member of any group of algae.

However, advanced oogamy is observed in several other cases such as in brown algae (*Sargassum*, *Fucus*) and red algae. In red algae, reproductive organs are highly specialised and *Polysiphonia* is at the top of the list where even male gametes are non-motile.

Thus, the sex evolution followed the path of sex organ differentiation with the retention of motility and smaller size in male gametes. The female gamete shows increase in size and nutritive capacity with the loss of motility. The non-motile nature of female gamete is a device to economise energy and to accumulate maximum food. The course of sex evolution in algae occurred in several independent lines without any particular phylogenetic line. The simplest alga *Chlamydomonas* itself shows iso- aniso- and oogamy and serves an adequate example in illustration whereas *Volvox* line evolution proceeds from *Gonium* to *Pandorina*, *Eudorina* and finally to *Volvox*. No exact course of sex evolution is possible to trace.

Economic Importance of Algae

It may be studied under two separate headings.

Beneficial Aspects

(1) Algae as Food

(a) **Utilisation of algae as fodder.** Sea weeds, especially brown algae, are used as food for domestic animals in different parts of the world and their wide scale usage centres in countries like Norway, Scotland, France, Great Britain, Scandinavia, America, New Zealand etc. Some of them have even industrialised the process and have established sea weeds factories for production of feed for cattle, poultry and piggery. There are reports from Denmark and USA about dried sea weeds usage such as of *Pelvetia* as cattle food enhanced the ten percent milk yield of cattle and egg laying capacity of poultry. Marine algae have immense value for their minerals and vitamins.

Pelvetia canaliculata is used as food for young live-stock. *Sargassum*, *Fucus* and *Laminaria* are eaten by cattle in Scotland and Island. *Macrocystis* spp. and other helps being rich in vitamins A and E have served as cattle fodder.

(b) **Utilisation of marine algae as human food.** Marine algae are of considerable importance in food value. They are often mixed with rice and fish and serve as base for soups, condiments and eaten alone as salads. The edible forms are called *Limu* in Hawaii, *Tsao* in China and *Rimu* in Tahiti.

The national diet of Japan consisting of rice, fish and sea weeds is looked upon as reason for good health, high degree of intelligence, usual artistic, literary and scientific attainment of Japanese people.

Red alga, *Porphyra* forms a common item of human diet and is used in food called "Amanoria or Nori" in Japan and Korea, 'Tsats' ai' in China and 'Sloke' in Great Britain. It is used as salts in meats prepared in North Pacific Indians and is grilled on toasts in Great Britain. 'Nori' a famous Japanese paste, is extremely rich in vitamins B and C. It is cultivated on Bamboo frames in water.

In Scotland and Ireland, *Ulva lactuca* is used as salad and soups. *Laminaria saccharina* and *Rhodomenia palmata* are used as food and *Porphyra* is considered to be a tasteful culinary dish.

Some products of algae such as gelatinous carbohydrates from *Gigartinia stellata* and *Chondrus crispus* are used as puddings, eaten with milk or some times mixed with fruits or used as stabiliser in ice creams.

(c) **Utilisation of fresh water algae as human food.** *Chlorella*, a future hope of modern world as food supplement, is cultivated in pilot plants and industrially in Japan, America and China. Its growth is rapid with an output of hundred pounds per acre per day or 17.5 tons per acre in a year. The algae has been used as a flour for making crackers and biscuits on experimental basis. *Chlorella* is rich in protein and carbohydrates.

Blue green algae are little known for food value, though a terrestrial form of *Nostoc* is used in China, Java and in Equador. *Nostoc commune* is eaten as a food and is called 'yuyucho'.

(d) **Utilisation of algae as fish food.** Algae serve as primary food for fishes and other small aquatic animals. Such algae may be both plankton or an attached form in the sea as well as in fresh water. *Zebrasoma flavescens*, the surgeon fish feeds on red alga *Amansia glomerata* and brown alga *Ectocarpus*. In Philippine Islands, fish food culture has been maintained in which blue green gelatinous filamentous alga, *Lyngbya aestuarii*, serves as baby food for fry. After three months these fishes are removed to the ponds containing *Cladophora* and *Chaetophora*. A few months later they are transferred to the pond in which green alga, *Enteromorpha* grows.

In fresh water lakes and ponds, *Oedogonium*, *Spirogyra*, *Microspora* *Ulothrix*, *Cladophora*, *Pithophora* etc. serve directly as fish food.

Algae are asset to fish life since oxygen is vital for their existence and carbon dioxide in certain concentration acts lethal to fishes. By photosynthetic process algae liberate oxygen in water and remove carbon dioxide.

(2) Algae in Industries

(a) **Alginic acid derivatives.** Alginic acid derivatives are extracted from the members of Phaeophyceae such as *Laminaria*, *Ascophyllum*, *Macrocystis*, *Ecklonia*, *Lessonia*, *Durvillea*. In composition, alginic acid is similar to that of cellulose and pectic acids, consisting of a long unbranched chain of (β-d-mannuronic acid joined by 1:4 glycosidic linkages. Alginic acid and its Ca, Al, Zn, Co, Cr, Fe salts are insoluble in water. The soluble alginates behave as colloids. The alginates are non-toxic, highly viscous and readily form gels.

Alginates are used as thickness in food industry for filling creams, in cosmetics as hand creams, in textile industries as printing pastes, in rubber industry in latex production, as emulsifiers in ice creams, synthetic creams, processed cheese, pharmaceutical emulsions, polishes, emulsion paints, as gelling agent in confectionary and meat jellies, as dental impression powder, in paper industry for sulphur film and glazes on ceramics. Alginates are also used as gel in the freezing of fish, antibiotics (Aureomycin) etc.

(b) **Carrageenin.** It is the most famous carbohydrate mucilage named after Irish village Carrageenin. The gelatinous carbohydrates are variously used with puddings, eaten with milk or mixed with fruit and even in ice cream. It is also used as clearing agents in beer preparation.

It is extracted from red alga *Chondrus crispus*—"Irish moss" and to a lesser extent from *Gigartina* spp. The compound is a cell wall polysaccharide complex of D-galactose-3, 6-anhydro-D-galactose and monoesterified sulphuric acid and two of its major components have been recognised.

(i) Kappa carrageenin, i.e. D-galactose-4-sulphate-3, 6-anhydro-D-galactose, and

(ii) Lambda carrageenin, i.e. D-galactose sulphate.

These compounds are used like alginates in food, textile, pharmaceutical, leather and brewing industries.

(c) **Agar** Dried gel-like non nitrogenous extract from red algae, agar is used as a medium in the cultures of bacteria, fungi and algae and also in numerous industrial processes. Dried agar is insoluble in cold water but soluble in hot water. A dilute solution (1-2 per cent) remains liquid down to a temperature varying between 35 to 58°C. Until 1939, Japan was the largest producer of agar which was largely prepared from *Gelidium* but now several other countries dominate. The prominence in agar producing countries has been gained by Japan, USA, Australia, Chile, S. Africa, New Zealand, N. America, British Isles and Baltic countries. Algae such as *Campylodictyon*, *Pterocladia*, *Gracilaria*, *Eucheuma*, *Chondrus*, *Gigartina*, *Phyllophora*, *Furcellaria* are used for extraction.

Agar from *Gelidium cartilagineum* is known to consist of a chain of alternating D-galactose and 3:6 anhydro-L-galactose residue with a half ester sulphate one about every tenth unit of the galactose. Pyruvic acid, Uronic acid, Polysaccharides like agaropectin and agarose are also known to occur in agar agar.

Besides as a medium for culture of microorganisms, agar has also been used in the food bakery, cosmetics, pharmaceuticals, leather, textile industries, in confectionary, dental impression mold, meat packing and as laxatives and emulsifiers.

(d) **Iodine and other compounds.** Brown algae like *Laminaria digitata*, *Ecklonia*, *Eisenia* and *Fucus* spp. are largely known for the extraction of Iodine.

(e) **Funori and funorin.** Japan prepares a sizing agent and glue for textile called *Funori* from *Gloeopeltis furcata* and for other inferior products algae like *Iridaea*, *Gratilauia*, *Chondrus*, and *Ahnfeldtia* are used. The composition of funori is similar to that of agar but it misses sulphate ester groups.

(f) **Diatomite.** Diatoms and their large sedimentary deposits "diatomaceous earth" are quite useful in industry. Diatomite is used in industrial filtration processes, sugar refining and brewing industries. For the removal of waste mycelium in the production of antibiotics it is used as filter and is also used as an absorbent for nitroglycerine in the manufacture of dynamite. Diatomaceous earth is also used as a filter in paint and plastic industries. It also acts as a catalyst in several industries these days.

(3) Medicine and Antibiotics

(a) **Medicines.** Because of high iodine content brown algae are used in various goiter medicines, either mixed or directly as a powder. *Palcato*, a medicine from *Sargassum* and members of *Laminariales*, is used as a goiter check by South Americans. It is also used for other glandular troubles.

A diet including agar has been used to cure prolapsed stomach. The distended stomach, after drinking a few glass of water regains normal position. *Gelidium* is used for other stomach disorders and for heat induced illness. Several kinds of pharmaceutical products such as pills and ointments and various laxatives are prepared from agar.

Sodium laminarin sulphate and fucoidin are used as anticoagulants but carrageenin acts as blood coagulant. Insect diseases to human beings (venomous diseases) are treated with extract from *Alsidium*, *Corallina*, *Codium*, *Durvillea* and *Digenia*. China, Chile and Japan use algae for treatment of kidney, bladder and lung diseases. Because of swelling property of laminaries, they find use in child's birth for the expansion of cervix.

Laminaria stipites are used as surgical tool in the opening of wound owing to its property of gentle swelling subject to moisture exposure.

Agar serves as medium for the culture of various organisms such as bacteria of medicinal importance. Antihelminthic medicines, *Tes—Ko—Tso* used in South China are prepared from *Digenia simplex*. An odourless, half sweet to sugar, powder of mannitol serves as supplement for sugar requirements of diabetic patients without harmful effects.

(b) Antibiotics. A new line of using some algae in the manufacture of antibiotics is in primary stage. *Chlorellin* is extracted from *Chlorella* which inhibits growth of certain bacteria and a few algae. *Nitzschia palea* is claimed to reduce the growth of bacterium *Escherichia coli*. Antibiotic properties from *Rhodospira rubra*, *Ascophyllum nodosum*, *Halidrys*, *Pelvetia* etc. have been reported. *Microcystis* is popularly known for its inhibitory action to *Staphylococcus* and *Clostridium* and zooplanktons such as *Daphnia* and *Cyclops*.

(4) Water Purification

In water reservoirs the larger growth of algae creates great nuisance but lesser growth of algae acts as biological filters by forming a microzone on the sand surface which together with bacteria and fungi forms a mucilage layer. By this microzone, harmful bacteria are trapped and water also gets aerated.

(5) Sewage Disposal

The presence of algae facilitates oxygenation of sewage to a great extent. The algae known to grow in sewage are *Euglena*, *Chlorella*, *Scenedesmus*, *Chlamydomonas*, *Pyrobotrys*, *Microactinium* etc. Silva and Papenfuss (1953) reported mainly Chlorococcales, Volvocales and Euglenophyceae from sewage waters. Algae form a surface film on sewage disposals which supplies oxygen and utilises nutrients to break down sewage.

(6) Uptake of Radioactive Wastes

Observation on surface absorption and uptake of radioactive elements by certain algae are also known. Meagre records available can be enumerated as follows:

Name	Element	Reference
<i>Porphyra lucinata</i>	Ru ¹⁰⁶	(Jones 1960)
<i>Chlorella</i> and <i>Euglena</i>	Cs ¹³⁷	Williams (1960)
<i>Scenedesmus quadricauda</i> , <i>Cladophora glomerata</i> , <i>Spirogyra</i> , spp.	Isotopes of Ca, Co, Fe, Rb, S, Zn, Sr	Gileva (1960)

(7) Land Reclamation

Algae such as *Nostoc*, *Scytonema* and *Anabaena* grow as thick stratum on surface of saline usar soils during the rainy season. Singh (1950) reported that they have capacity to reclaim usar soils.

Sometimes acting as binding agent algae reduce dangers of erosion of distributed or burnt heath soils by the rapid growth of forms such as *Hormidium*, *Zygogonium* (Chlorophyceae), *Chroococcus*, *Phormidium*, (Cyanophyceae) etc.

In North India, the soil is actually ploughed to encourage the algal growth before raising rice crop.

(9) Algae as Source of Growth Substances

The presence of growth promoting substances and their potentialities in stimulating rice yield and altering the quality of the grains have been recently explored. Shukla (1967) pointed out that the presoaking seed treatments with *Phormidium tenue* not only result in sturdier, intensely green plants, profuse tillering and increased height but also results in multiple rice yields. The protein contents of grains of treated plants also increases.

(9) Lens Paper

Japanese use *Spirogyra* in the manufacture of lens paper used for cleaning optical articles.

Harmful Aspects

(1) Death of Fishes

A compound toxin produced by certain algae proves fatal to fishes e.g. the growth of *Aphanizomenon* and *Microcystis aeruginosa* in fish ponds are poisonous to *Crappis perch* and *Gambusici* respectively. Certain Dinoflagellates are toxic not only to fishes but also to human beings. Blooming species of *Gymnodinium* produce enough toxin to kill fish within several square miles of Gulf of Mexico and in laboratory experiment the alga has been found lethal to 23 different species of test fishes.

Besides, the production of toxin, the side products of decay such as hydroxyl amine from algal protein exercise lethal effects on fishes. Algae also cause suffocation of fishes by choking their gills.

(2) Death of Animals

A few blue green algae such as *Microcystis*, *Anabaena*, *Nodularia*, *Gloeotrichia* and *Aphanizomenon* produce exotoxin and endotoxin causing death of animals, horses, cattle, sheep etc. The toxic capacity of algae is lost with their death. Death of animals within 1 to 24 hours after drinking the water contaminated with algae has also been reported. In laboratory experiments death due to intravenous, intraperitoneal and oral treatments occurred, some times within three minutes.

Besides death, the harmful effects of algae to cattles may bring about loss of weight, weakness, liver pathology, abortion etc. Some pigments of algae (phycocyanin) are sensitive to light and when they enter in the blood capillaries of cattle causes an internal burning and peeling of the skin.

The toxin from *Microcystis aeruginosa* is a cyclic polypeptide which includes about ten amino acids; some of them are D-serine, L-ornithine, aspartic acid and glutonic acid. Toxin results in enlargement of liver, failure of blood to clot and spleen congestion. Algal toxin is far more toxic than gramicidins or bacitracin and slightly less toxic than the poisons extracted from the mushroom *Amanita phalloides* (Konst et al, 1965).

The commonly known toxic algae are *Anabaena flos-aquae*, *A. circinalis*, *A. inaequalis*, *Aphanizomenon flos-aquae*, *Aphanothece nidulans*, *Gloeotrichia* spp., *Microcystis aeruginosa*, *M. toxica*, *Lyngbya* sp. *Nodularia spumigera*, *Scenedesmus* sp., *Chlorella* sp., *Gymnodinium brevis* etc.

(3) Death of Human Beings

Several cases of human death have been reported as a result of indirect consumption of dinoflagellates through fishes which have eaten them. *Gonyaulax* and other dinoflagellates when eaten with shell fish are known to produce several types of diseases. There are reports of paralysis, liver pathology, respiratory failure or death within 2 to 12 hours after eating *G. catanella*, *Gymnodinium brevis*, and *G. flavum*, when eaten with fish cause respiratory ailments.

The toxin compound of *Gonyaulax* is referred to as ichthyosarcodin with a chemical formula $C_{16}H_{31}NO_{16}$. Symptoms produced by such toxins are dizziness, nervous disorders and death within 30 minutes and 24 hours, respectively. A well known historical event is known when the whole company of Japanese soldiers in the South Pacific was killed from poisonous fish indirect consumption by accident during World War-II.

(4) Problems of Water Purification, Supply and Pollution

In storage water reservoirs, the algae badly effect in two ways. Firstly the immense growth of algae and their decomposition products produce a bad odour and secondly, they cause interference in water filtration. Such unwanted growth of algae is of common occurrence in low land reservoirs and slow running of rivers.

The water in the reservoirs is filtered through sand filters which are clogged due to overwhelming growth of algae. In this second way algae cause a big nuisance and create a serious problem. Under such conditions water has to be back-washed or upper sand layer has to be removed to take off algae. Diatoms (*Melosira*, *Cyclotella*, *Asterionella*, *Fragillaria*, and *Stephanodiscus* etc.), blue green algae (*Oscillatoria*, *Aphanizomenon*, *Anabaena*, *Microcystis* etc.) and some algae of other groups (*Chlamydomonas* and *Ceratium*) are commonly known to cause difficulty during filtration. Larger forms create difficulty by their accumulation in fast filters whereas smaller forms block the sand filters. Brook (1954) observed that old beds were predominately disturbed by non-motile algae such as *Scenedesmus quadricauda*.

To meet the problem, various chemicals such as low concentration of Copper sulphate (for *Spirogyra*, *Lyngbya*, *Phormidium*), 2-3-Dichloronaphthoquinone (for blue green algae forming water blooms) and Phenanthraquinone (for several bloom species and *Chlorella*) have been suggested to check the growth of these forms. The chlorination process at different intervals is also exercised in reservoirs but large doses of chlorine encourage the growth of smaller green algae.

The intensive growth of algae imparts bad and offensive smell and entire reservoirs become unsuitable. Besides several algae such as *Spirogyra*, *Pithophora*, *Rhizoclonium*, *Basidiella*, *Hydrodictyon* are known to choke the supply drains and small irrigation canals.

The pollution caused by algae is well known. They produce effective problems in water supply and purification and become obnoxious in water reservoirs, rivers and oceans.

(5) Damage to Salt by Blue-green Algae

India suffers an enough serious problem of blue-green algae causing threat of enormous loss to Indian Government revenue by affecting the quality of salt in Sambhar lake in Rajasthan. Algae produce an offensive smell, impart pink rust red colour to the salt and turn brine into a gelatinous fluid making it impossible to develop crystals. In Mandapam, Pillai (1955) observed the complete spoilage of lagoon vegetation due to excessive growth of algae. Here *Phormidium* grows even when the salinity of water exceeded 60 parts per thousand.

(6) Damage to Building by Blue-green Algae

The growth of blue-green algae on moist wall during rainy season and ultimately becoming permanent, spoil the walls of buildings all over the world. In tropics, common algae causing damage to buildings are *Scyionema*, *Tolypothrix*, and *Chroococcum* etc.

(7) Accidents due to Blue-Green Algae

Thick intensive growth of blue-green algae make the ground surface extremely slippery and result in human beings get slipped or in violent accidents of cattle.

PART – V**BRYOPHYTA**

0. Main Examination Questions

NOTE: Given the recent trends in the examination, candidates are advised to attempt each question in about 200 – 250 words.

1. Bryophytes are successful as land plants, but they could not become the dominant flora of the land habit. Explain.
2. How is the alternation of generations seen in thallophytes different from that seen in Bryophytes?
3. Enumerate the common characters shared by Liverworts, Hornworts, and Mosses. How can you differentiate between them?
4. How would you differentiate between a leafy liverwort and a moss?
5. Range of sporophytes within the mosses.
6. Economic, ecological, and medicinal importance of bryophytes.
7. Adaptations of land life as evident in Bryophytes.
8. Why were bryophytes not successful as land plants?
9. Give a short account of a moss protonema.
10. Three most important characters, which differentiate Bryophytes from the Pteridophytes.
11. In what ways does *Anthoceros* show similarities with the Pteridophytes?
12. Vegetative propagation methods in Liverworts.
13. Elaters. –
14. Life history of *Sphagnum*. –
15. Evolutionary trends in the gametophytes of Bryophytes.
16. Retrogressive development of sporophytes in Bryophytes.
17. Leaf structure of *Polytrichum*.
18. Bryophytes as indicator plants.
19. Comparison between the sporophytes of *Anthoceros* and *Polytrichum*.
20. Differentiate between a liverwort and a moss.
21. Sporophyte of *Funaria*.
22. Economic importance of Bryophytes.
23. Indirect uses of Bryophytes
24. Write about the unique features of the sporophyte of *Anthoceros*. Briefly point out the evolutionary trends in the sporophytes in Bryophytes.
25. What is the ecological significance of the bryophytes?
26. Write about *Sphagnum* under the following headings.
 - Morphology
 - Reproduction
 - Ecology
 - Economic importance
 - Relationships
27. Give the characteristics of Jungermanniales. Why are they included in Liverworts?
28. Hornworts as a synthetic group.
29. Vegetative reproduction in Bryophytes
30. Evolutionary trends in Sporophytes
31. Evolutionary trends of sporophytes in Bryophytes.
32. What is apogamy? Also, describe the structure and arrangement of sex organs in Bryophytes.

1. Introduction to the Bryophytes

The bryophytes are those embryophytic land plants that are non-vascular: they have tissue differentiation and enclosed reproductive systems, but they lack vascular tissue that circulates liquids. They neither flower nor produce seeds, and reproduce via spores. They are also called the amphibians of the plant world, due to a preference for moist habitat and dependence on water for fertilization despite being land plants.

The Bryophytes include 3 groups:

1. liverworts
2. mosses
3. hornworts

Distribution

Bryophytes are generally small but form a striking part of the vegetation in cooler and moist northern and southern latitudes and extremely humid climates of both temperate and tropical regions. Bryophytes sometimes carpet the forest floor with vivid greens and regions. They also form extensive peat lands with hummocks and depressions of rich greens, browns, and reds. Trees may be sheathed in bryophytes that extend up the trunks and encircle branches. In foggy forests the tree branches are often draped with pendent ropes of bryophytes. These essentially terrestrial plants have achieved the greatest structural diversity shown by the gametophyte.

Basic Diversity

As mentioned above, the division Bryophyta is divided into three classes:

1. Hepaticae (liverworts and scale mosses)
2. Anthocerotae (hornworts)
3. Musci (mosses).

Although these names are used traditionally, the International Rules of Botanical Nomenclature would recommend Hepaticopsida, Anthocerotopsida, and Bryopsida respectively.

Some modern authors like Linda Graham, Kenrick and Crane and others treat each of these classes as a division because it is now accepted that three groups of Bryophyta have arisen independently (parallelly) from a chaetophoralean stock in late Ordovician. Hence, according to the currently accepted view, the bryophytes do not form a monophyletic group but consist of three groups, the Hepaticophyta (liverworts), Anthocerotophyta (hornworts), and Bryophyta (mosses).

However, we can not deny that these plants share many fundamental characteristics among themselves; some of them are outlined below.

Basic features

1. They are small green land plants, possess chlorophyll A and B, starch, cellulose walls, and sometimes possess a cuticle.
2. The life cycle always has alternation of generations.
3. The dominant and ecologically the persistent generation is always the gametophyte.
4. The sporophyte is short lived to annual.
5. The sporophyte, although photosynthetic through most of its life span preceding spore dispersal, is always attached to the gametophyte and is at least partially dependent on it. The base of the sporophyte (the foot) penetrates the tissue of the gametophyte.
6. The sporophyte is unbranched and produces a single terminal sporangium.
7. The spore coat is cutinized, and they are generally disseminated through the air.
8. The sporophyte and gametophyte possess no lignified tissue.
9. The sporophyte and gametophyte possess no vascular tissue.
10. The gametophyte is generally perennial; it often consists of two stages:
 - i. juvenile usually filamentous phase (protonema); and
 - ii. a more complex phase (the gametophore) that actually produces the sex organs.

11. The male gametes (sperms) are biflagellate with whiplash flagella and must reach the egg via a water film. They are produced in an antheridium that is a stalked sac. This sac is composed of a sterile unistratose (one cell in thickness) jacket enclosing innumerable cells. Each of which produces a sperm.
12. The female sex organ (archegonium) is flask shaped; the neck of the flask is unistratose, while the lower expanded portion (venter) may be multistratose; each archegonium encloses a single egg.
13. Growth of the gametophore is by a single apical cell, rather than by meristematic tissue.
14. Bryophytes are generally small, with the sporophyte usually no more than 3 cm tall and the gametophore usually less than 10 cm tall, although erect forms may exceed 20 cm, and reclining, aquatic, or hanging forms may reach a meter in length.

Reproduction

The principal mode of reproduction is sexual, that takes place in the gametophytic generation.

It is typical of bryophytes to produce large, multicellular sex organs. Many bryophytes are unisexual [or sexually dioicous]. In bryophytes male sex organs, called antheridia, are produced singly or in clusters and female sex organs, the archegonia, are produced in similar fashion. Numerous motile sperm are produced by mitosis inside the brightly colored, club-shaped antheridia while a single egg develops in the base of each vase-shaped archegonium.

As the sperm mature, the antheridium swells and bursts open. Drops of rain water falling into the cluster of open antheridia splash the sperm to near-by females. Beating their two whiplash flagellae, the sperm are able to move short distances in the water film that covers the plants to the open necks of the archegonia. Slimy mucilage secretions in the archegonial neck help pull the sperm downward to the egg. The closely packed arrangement of the individual bryophytic plants greatly facilitates fertilization. Rain forest bryophytes that hang in long festoons from the trees rely on torrential winds with the rain to transport their sperm from tree to tree, while the small pygmy bryophytes of exposed, ephemeral habitats depend on the drops of morning dew to move their sperm.

Regardless of where they grow, all bryophytes require water for sperm dispersal and subsequent fertilization.

Embryonic growth of the sporophyte begins within the archegonium soon after fertilization. At its base, or foot, the growing embryo forms a nutrient transfer zone, or placenta, with the gametophyte. Both organic nutrients and water move from the gametophyte into the sporophyte as it continues to grow. In bryophytes the sporophyte has three parts: 1. Foot 2. Stalk, or Seta and 3. Capsule. Depending on groups, these parts attain differential growth.

Within the capsule, water-resistant spores are formed by meiosis. The mature capsule gets to dry and break open to release the spores into the drying winds. The spores can travel great distances on the winds, even moving between continents on the jet streams. Their walls are highly protective, allowing some spores to remain viable for up to 40 years. If the spore lands in a suitable, moist habitat, germination will begin the cycle all over again.

The Figure below shows the alternation of generations, predominance of the gametophyte and the dependency of the sporophyte over the gametophyte in Bryophyta. The life cycle chosen here to display these principles is of *Polytrichum* (a moss) but the principles apply to all the Bryophytes.

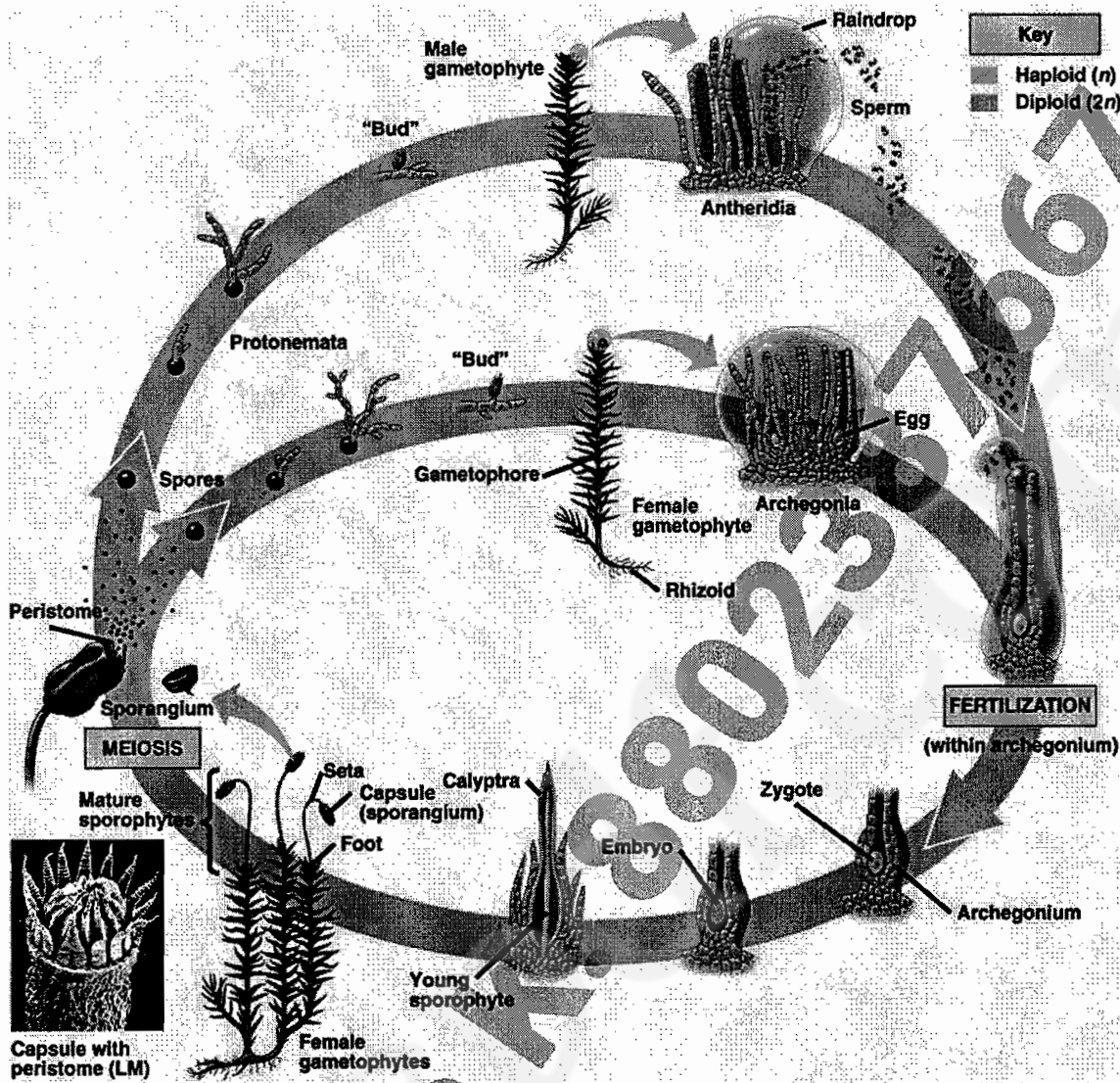


Figure 1: Life cycle of Bryophytes

2. Diversity of the Bryophytes

The division Bryophyta has been divided into three classes in traditional systems:

1. Liverworts and scale mosses - **Hepaticopsida**
2. Hornworts - **Anthocerotopsida**
3. Mosses - **Bryopsida**

Modern authors like Linda Graham, Kenrick and Crane and others treat each of these classes as a division because it is now accepted that **three groups of Bryophyta have arisen independently (parallely) from a chaetophoralean stock in late Ordovician**. Hence, according to the currently accepted view, the bryophytes do not form a monophyletic group but consist of three divisions,

1. **Hepaticophyta** (liverworts)
2. **Anthocerotophyta** (hornworts)
3. **Bryophyta** (mosses)

Liverworts

The liverworts include the thallose liverworts and leafy liverworts or "scale mosses." The class consists of approximately 8,000 species. The detailed evolutionary trends are clearly represented by the seven orders:

1. Order Takakiales (Controversial)
2. Order Calobryales
3. Order Jungermanniales
4. Order Metzgeriales
5. Order Sphaerocarpaceae
6. Order Monocleales
7. Order Marchantiales

These orders are based essentially on structure of the gametophyte, particularly the gametophore. The nature of the sporophyte is very similar among orders.

Distinctive features of liverworts

The hepatics possess a number of features that distinguish them from the mosses:

1. The "protonema" is usually reduced to two or three cells of the uniseriate germ tube, and the apical cell of the gametophore is differentiated early.
2. The protonematal phase produce no gemmae.
3. Liverwort gametophytes can be either leafy shoots or flattened thalli.
4. In the leafy forms, the leaves [strictly speaking the phyllids] are arranged on the stem in one ventral and two lateral rows or ranks, rather than in spirals like the mosses.
5. The leaves are mostly one cell layer thick throughout, never have a midvein and are usually divided into two or more parts called lobes.
6. The ventral leaves, which actually lie against the substrate, are usually much smaller than the lateral leaves and are hidden by the stem.
7. Leaf cells are commonly isodiametric and frequently possess trigones.
8. Anchoring rhizoids, which arise near the ventral leaves, are colorless and unicellular.
9. The flattened ribbon-like to leaf-like thallus of the thallose liverworts can be either simple or structurally differentiated into a system of dorsal air chambers and ventral storage tissues.
10. The leaves never show spiral arrangement.
11. Sex organs lack paraphyses among them, but mucilage filaments are usually present.
12. Gametophore cells often have complex oil bodies.
13. The jacket of the sporangium never has stomata.
14. The sporangium jacket is sometimes unistratose.
15. The sporangium usually opens by four longitudinal lines.
16. Even in the rare cases where there is an operculum, peristome teeth are absent.

17. Within the sporangium there are often sterile threadlike hygroscopic cells with helical wall thickenings; these are elaters; a columella is always absent.
18. The sporophyte usually produces a colorless seta of thin-walled cells and is held rigid by turgor pressure in the component cells.
19. The sporophyte generally persists for a very brief period after the spores are shed.
20. The calyptra is ruptured and remains at the base of the seta when the seta elongates rapidly and protects the maturing sporangium before seta elongation.
21. The seta elongates after the sporangium is completely differentiated with the included spores and elaters, and the seta is rarely a photosynthetic organ.
22. Generally all spores in a sporangium are shed at the same time.

The Figure 1 below shows the reproduction of liverworts.

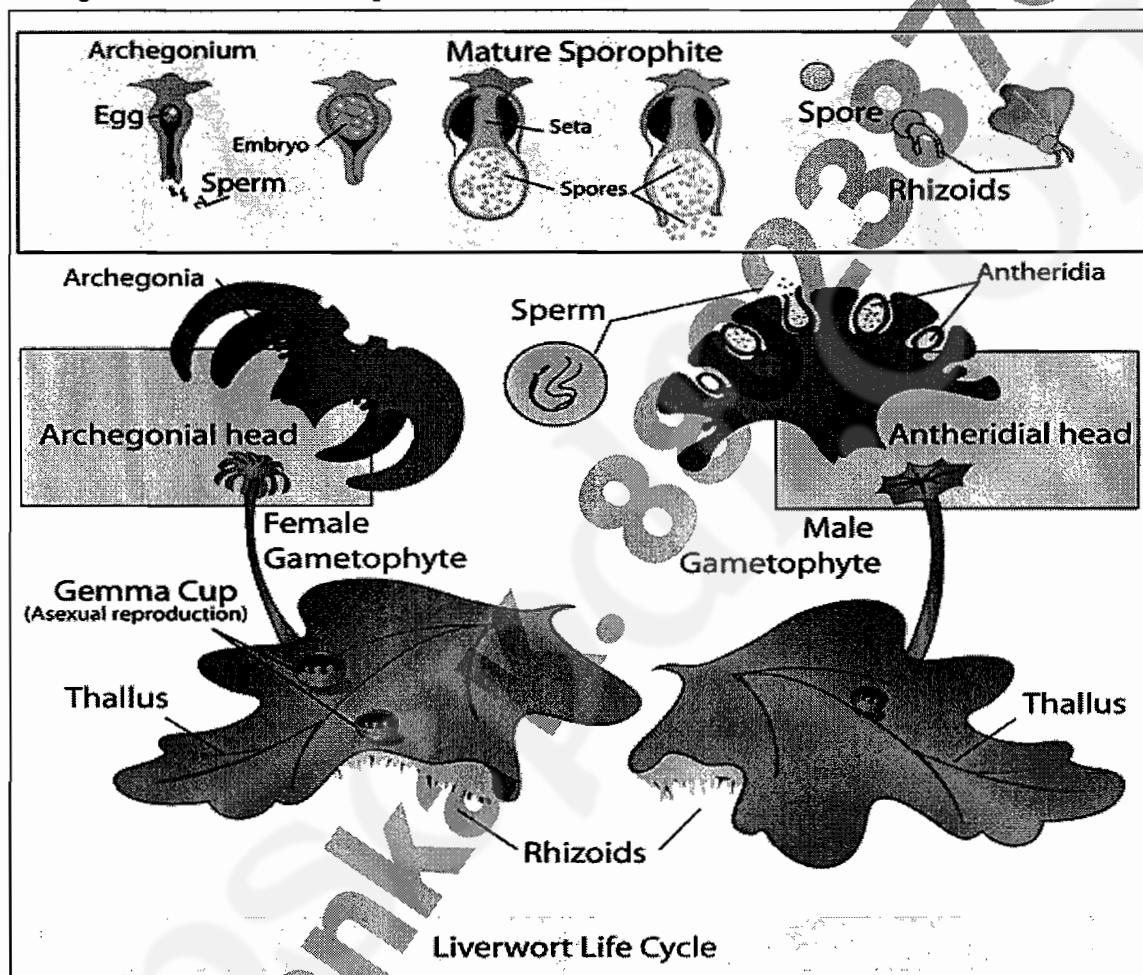


Figure 1: Liverwort reproduction

Mosses

Of the three phyla of bryophytes, greatest species diversity is found in the mosses, with up to 15,000 species recognized.

Mosses are generally the most conspicuous bryophytes in the vegetation. Mosses show greater structural diversity than other bryophyte classes. Mosses have seven subclasses:

1. Subclass Andreaeidae
2. Subclass Sphagnidae
3. Subclass Tetraphidae
4. Subclass Polytrichidae
5. Subclass Buxbaumiidae
6. Subclass Bryidae

7. Subclass Archidiidae

These subclasses are founded essentially on structure of the sporophyte.

Distinctive features of mosses

Several features distinguish mosses from other bryophytes:

1. The protonema is generally an extensive, branched filamentous phase of the life cycle. In it the cross walls are often oblique
2. Rhizoids are always multicellular and resemble the protonema, except that they lack chlorophyll and often have brownish pigmented walls
3. The protonema sometimes produces gemmae (= rhizoidal gemmae or 'tubers')
4. The gametophore is always leafy, and the leaves are generally radially arranged in more than three rows.
5. Antheridia and archegonia usually have sterile filaments (paraphyses) intermixed among them.
6. Leaves of the gametophore are unistratose for the most part, except at the multistratose midrib (costa). The costa may be single or multiple, or rarely absent.
7. Leaf cells are commonly elongate and rarely possess trigones.
8. Leaf cells have simple small oil bodies, if any
9. Leaves are rarely lobed.
10. The jacket of the sporangium generally has stomata.
11. The outer cells of the sporangium jacket lack transverse bar like thickening or nodular thickening.
12. The sporangium jacket is always multistratose.
13. The sporangium usually opens by means of an apical lid (operculum)
14. When the operculum falls loose, it usually exposes teeth (peristome teeth) that form a ring around the opening. These teeth are often hygroscopic.
15. Within the sporangium there is usually a central mass of sterile tissues (columella).
16. The sporangium is usually elevated on an elongate stalk (seta); the seta is usually wiry and made up mainly of thick walled cells.
17. The calyptra usually is torn loose from the gametophores by the elongating sporophyte and protects the tip.
18. The seta elongates before the sporangium is differentiated, and is a photosynthetic organ for an extended period during sporophyte development. The presence and shape of the calyptra influence the shape and differentiation of the sporangium.
19. Generally the spores are shed from the sporangium over an extended period.

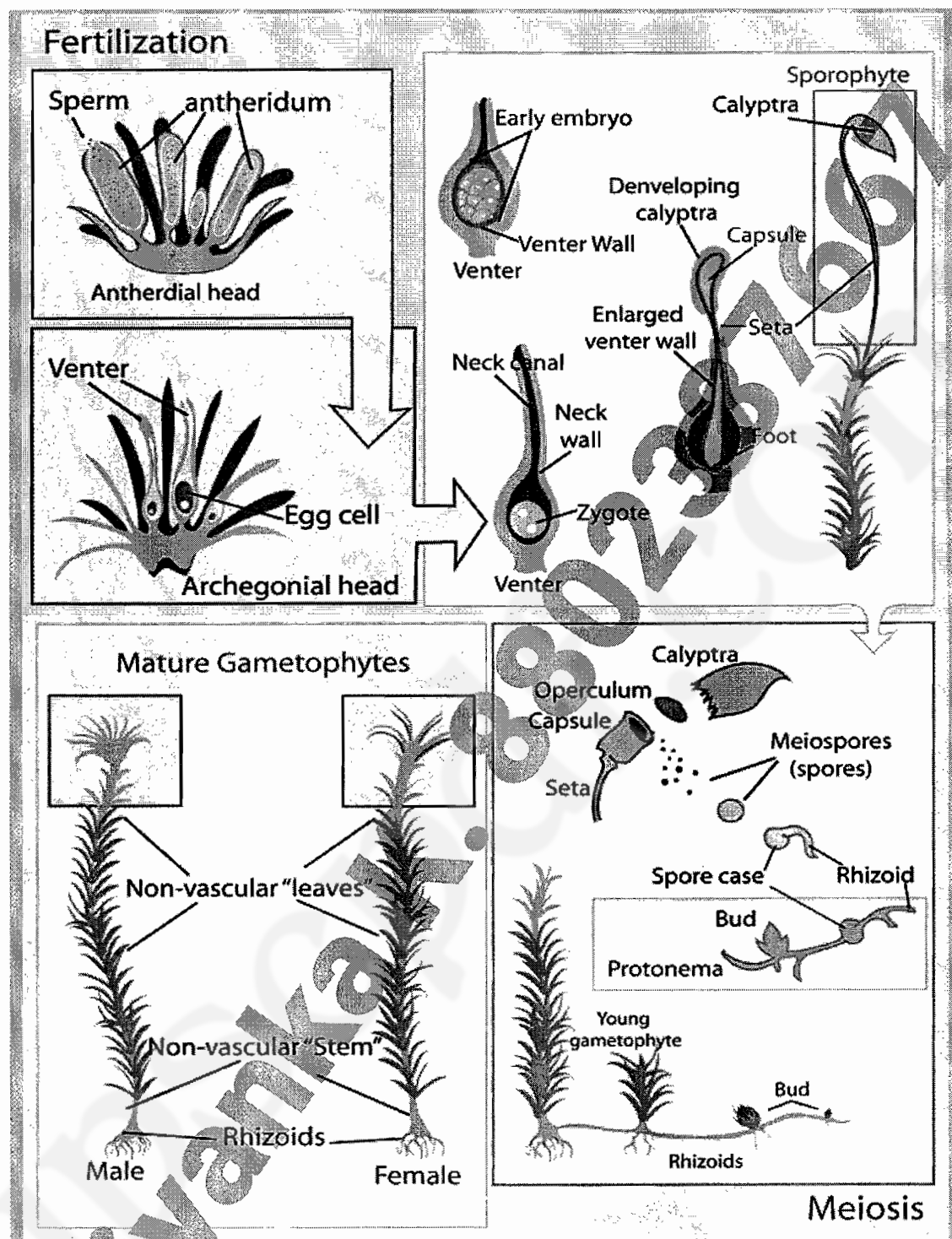


Figure 2: The life cycle of a moss

Hornworts

Hornworts get their name from their long, horn-shaped sporophytes. There are about 400 species of hornworts in the world. They are found in tropical forests and along streamsides. They are usually small and greenish-blue. They are long and narrow and have sporophytes at their tips.

Hornworts resemble some liverworts in having simple, unspecialized thalloid gametophytes, but they differ in many other characters.

The Hornworts form an isolated evolutionary line. Indeed some researchers consider them to be entirely independent from the bryophytes. The "horn" describes the sporophyte, a tapered cylinder that shows indeterminate growth in most species.

The class consists of at least four genera, although some researchers recognize six. These are in a single order Anthocerotales and are sometimes included in a single family, Anthocerotaceae, but some authors place *Notothylas* in its own family, Notothylaceae. Commonly recognized genera, besides *Notothylas* (ca. 13 species), are *Anthoceros* (ca. 250 species), *Megaceros* (ca. 46 species), and *Dendroceros* (ca. 51 species). *Phaeoceros* (ca. 30 species) and *Folioceros* (ca. 19 species) are sometimes segregated from *Anthoceros*.

Features that characterize the hornworts

1. A dorsiventrally flattened thallus commonly forms a rosette.
2. The thallus, composed of thin walled cells, is attached to the substratum by smooth rhizoids.
3. Each of the cells of the thallus usually contains a single large disc-shaped chloroplast, which frequently has an included pyrenoid.
4. The thallus often has mucilage filled cavities formed by breakdown of groups of cells; these cavities are often invaded by the blue green alga, *Nostoc*.
5. The thallus sometimes has ventral pores, resembling stomata in form (sometimes termed as slime pores).
6. The sex organs, although embedded in the upper layers of the thallus when mature, are formed from superficial cells.
7. Some thalli produce internal "tubers" that survive the unfavorable season.
8. Numerous antheridia often originate within a single antheridial chamber. Antheridia are discrete organs.
9. Archegonia are not discrete organs, but are represented by neck canal cells and an egg surrounded by essentially undifferentiated cells of the thallus.
10. The first division of the zygote is by a longitudinal line, thus differing from other bryophytes.
11. The sporophyte is always a tapered horn with no seta.
12. The sporophyte possesses a basal intercalary meristem and has indeterminate growth in most genera (except *Notothylas*).
13. The sporophyte usually grows throughout the favorable season, shedding spores at the apex and differentiating new spores from the intercalary meristem above the foot.
14. The young sporophyte is often protected by a thallus calyptra (sometimes termed an involucre) that elongates as the sporophyte elongates, and encloses it in a sleeve. Sometimes the apex is ruptured to form an apical calyptra while the remainder sheathes the base of the sporophyte.
15. The sporangium usually opens by one or two longitudinal lines; initially the split does not extend to the apex of the sporangium; the jacket walls are often hygroscopic.
16. The sporangium jacket is multistratose and frequently possesses stomata.
17. There is usually a cylindric columella in the sporangium.
18. The sporogenous layer overarches the columella.
19. There spores usually have Multicellular, somewhat hygroscopic elaters among them.

As apparent from the preceding list of features, the class is highly distinctive. Since it is an archegoniate plant, its basic design in gametophyte and sporophyte is extremely similar to many hepatics, and it shows numerous other bryophytic features, it is logical to include it with the Bryophyta.

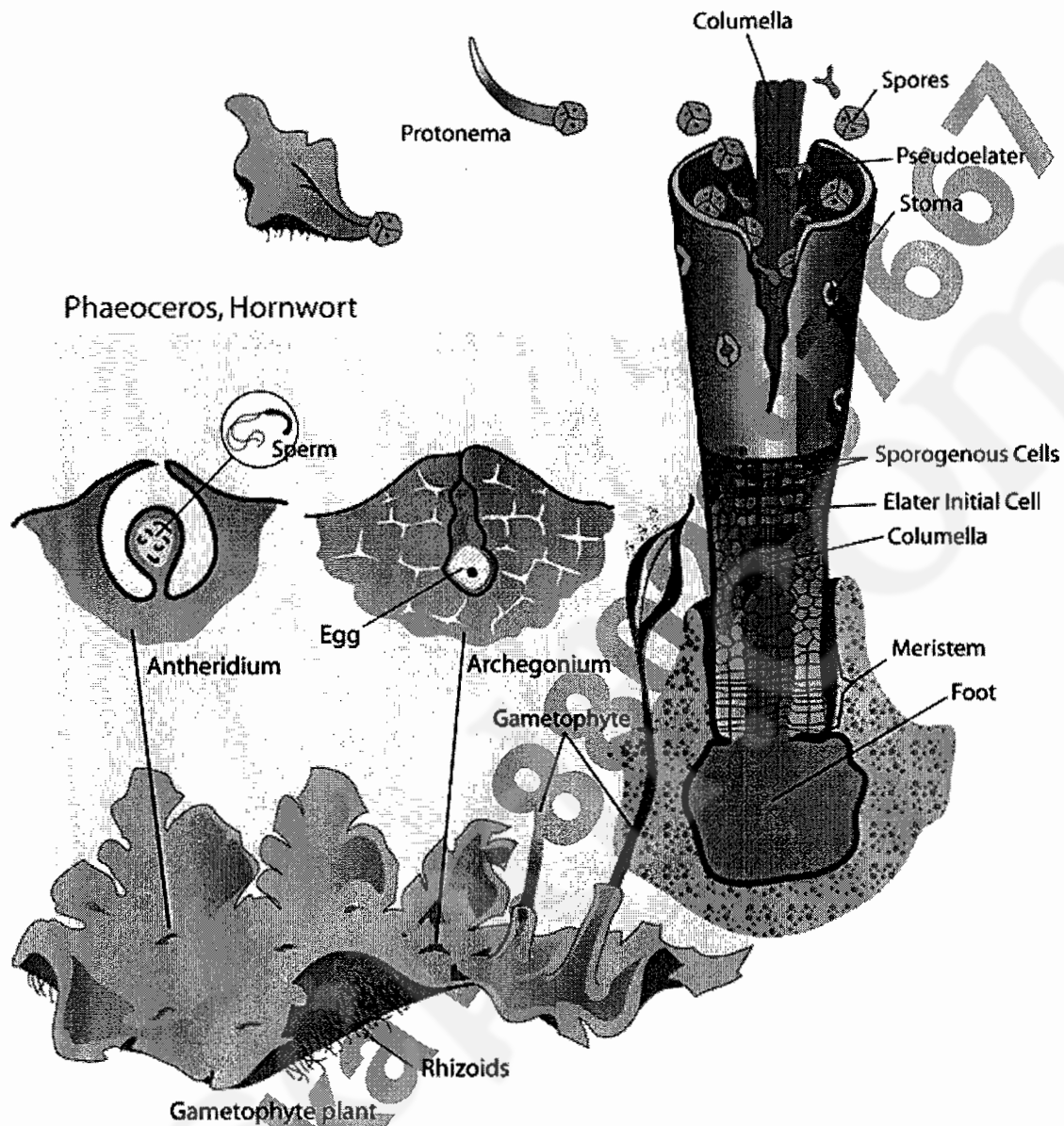


Figure 3: The life cycle of a hornwort

Comparison among the Mosses, Liverworts and Hornworts

Character	Mosses	Liverworts	Hornworts
Protonema	Filamentous, forming many buds	Globose, forming one bud	Globose, forming one bud
Gametophyte form	Leafy shoot	Leafy shoot or thallus; thallus simple or with air chambers	Simple thallus
Leaf arrangement	Leaves in spirals	Leaves in three rows	Not Applicable
Leaf form	Leaves undivided, midvein present	Leaves divided into 2+ lobes, no midvein	Not Applicable
Special organelles	None	Oil bodies	Single plastids with pyrenoids
Water conducting cells	Present in both gametophytes and sporophytes	Present only in a few simple thalloid forms	Absent
Rhizoids	Brown, multicellular	Hyaline, one-celled	Hyaline, one-celled
Gametangial position	Apical clusters	Apical clusters (leafy forms) or on upper surface of thallus	Sunken in thallus, scattered
Stomates	Present on sporophyte capsule	Absent in both generations	Present in both sporophyte and gametophyte
Seta	Photosynthetic, emergent from gametophyte early in development	Hyaline, elongating just prior to spore release	Absent
Capsule	Complex with operculum, theca and neck; of fixed size	Undifferentiated, spherical or elongate; of fixed size	Undifferentiated, horn-shaped; growing continuously from a basal meristem
Sterile cells in capsule	Columella	Spirally thickened elaters	Columella and pseudaelaters
Capsule dehiscence	At operculum and peristome teeth	Into 4 valves	Into 2 valves

Comparison between the Mosses and Leafy Liverworts

A leafy liverwort and a moss differ from each other in many fundamental ways in virtually all aspects of the life cycle.

IN THE ORIGIN OF THE GAMETOPHYTE

In a leafy liverwort

The development of gametophyte from a spore is a continuous process. The spore, on germination, produces a single sporeling that passes into an adult gametophyte plant. The protonema, when present, is small, and transitory.

In a moss

The development of gametophyte from a spore is not a continuous process. It passes through two distinct stages.

- **Juvenile stage:** From spore to protonema; the spore, on germination, produces an extensively branched green filamentous protonema. Such a protonema is conspicuous and relatively persistent.
- **Adult stage:** Development of a leafy shoot take place from the protonema; and usually a number of leafy gametophyte plants arise from it.

IN THE ADULT GAMETOPHYTIC PLANT

In a leafy liverwort

- The plant body is foliose yet it remains dorsiventral. Internally, there is no differentiation of the central conducting strand. *Takakia* and *Haplomitrium* are the only exceptions.
- Phyllids lack a midrib. The phyllid development growth is largely intercalary. Because of clear dorsiventral organization, the phyllid arrangement is well defined into lateral and ventral rows.
- The rhizoids are unicellular and unbranched.
- The development of sex organs starts at an intercalary position and not apical.

In a moss

- The gametophyte is radial in orientation. Anatomically, there is a differentiation of a central conducting strand.
- The phyllids cannot be clearly demarcated as lateral or ventral ones. Phyllids seem to be radially or spirally organized. Except in *Sphagnum*, a phyllid, as a rule, has a midrib. The development of phyllid is by the activity of an apical cell.
- The rhizoids are multicellular and branched. They contain characteristic oblique septa.
- The early development of sex organs is by the activity of a clearly defined apical cell. The sexual development is not intercalary.

IN THE SPOROPHYTIC PLANT

In a leafy liverwort

- The early growth of embryo is by successive transverse cleavage and thus intercalary.
- The hepatic seta is soft, pellucid and without any internal differentiation.
- Sporophyte breaks through the calyptra at a late stage (only when the spores are ripe) by the lengthening of the seta.
- The hepatic capsule is simple in organisation; it lacks the basal apophysis region and terminal operculum region of moss capsule and represents only the middle spore-producing theca region.
- The capsule lacks stomata on the capsule wall.
- There is no air space within.
- There is no annulus.
- The hepatic capsule lacks columella, the sterile tissue mass within the capsule.
- Elaters are generally present to facilitate spore dispersal.
- The entire endothecium is devoted to the formation of spores and elaters.

In a moss

- Except *Sphagnum*, the early embryo growth is biapical.
- Except *Sphagnum* and *Andreaea*, the moss seta is fairly long and tough with a well developed hypodermis and a central strand.
- Except *Sphagnum*, sporophyte breaks through the calyptra at an early stage by the lengthening of the seta.

- The moss capsule is highly organized both externally as well as internally. Externally it is differentiated into:
 - The basal photosynthetic *apophysis region*.
 - Middle fertile *theca region* and
 - Terminal *operculum region*.
- Except *Sphagnum* and *Andreaea*, the moss capsule has stomata on the capsule wall. There are one or two air spaces as well.
- Annulus is present in most of the moss capsules. It assists in the detachment of lid.
- Columella is as a rule, present in moss capsules.
- The elaters, as a rule, are absent. In some genera pseudoelaters may be present, but never true elaters. The dispersal of spores is controlled by the peristome.

Except *Sphagnum*, the outermost layer of endothecium is devoted to the formation of spores and the rest produces the columella.

Detailed systematics of the bryophytes (characters of individual groups)

There are about 20,000 species of Bryophytes. At one time, the bryophytes were placed in a single division, intermediate in position between algae and vascular plants. Modern studies of cell ultra structure and molecular biology, however, confirm that bryophytes comprise three separate evolutionary lineages, which are today recognized as

- Mosses (division Bryophyta)
- Liverworts (division Hepaticophyta)
- Hornworts (division Anthocerotophyta)

The classification of bryophytes presented here reflects main evolutionary lines and the traditional systematics. These seem best illustrated at the order level. Those orders that are considered to be most generalized are treated first; and those most specialized, last.

Classification of the liverworts leans heavily on gametophyte structure, with sporophyte structure providing additional evidence of relationships. In the hornworts and mosses, the structure of the sporophyte, especially the sporangium, is important in distinguishing the main evolutionary lines, while gametophytic features provide the details for distinguishing genera and species.

Division Hepaticophyta or Class Hepatopsida (or Hepaticae; liverworts)

Protonema generally reduced to a few cells, with gametophore differentiated early after spore germination; rhizoids unicellular; gametophore leafy or thallose and generally flattened; sex organs lacking paraphyses; leaves lacking true midrib; leaf cells often with corner thickenings; complex oil bodies often in cells of gametophore; sporangium jacket lacking stomata, and often with transverse thickenings in cellwalls; sporangium usually opening by longitudinal lines; sporangium releasing all spores and elaters at the time it opens; calyptra remaining at base when seta elongates.

1. Order Takakiales

Leaves cylindrical and irregularly but radially arranged on an erect shoot that arises from a subterranean, colourless, rootlike system; sex organs lateral but near shoot apex; rhizoids absent; sporophytes unknown; Southeast Asian and northwestern American distribution; a single genus, *Takakia*, with two species.

2. Order Calobryales

Leaves flattened and in three rows on an erect shoot arising from a colourless, subterranean, rootlike system that lacks rhizoids; sex organs lateral but near shoot apices; sporophytes with elongate seta; sporangium elongate, with elaters and thickenings on the jacket cell walls; opening by 1–4 longitudinal lines; mainly of mid-latitudes, most species in the Australasian and Indo-Malayan region; 2 genera, *Haplomitrium* (12 species) and *Steereomitrium* (1 species).

3. Order Metzgeriales

Thallose, with the thallus mainly of uniformly thickened cell walls, usually reclining but sometimes erect; branching varies from forked to regularly pinnate or irregular; smooth rhizoids on the undersurface; sex organs lateral; sporophytes with elongate seta; sporangia spherical to elongate, with elaters and thickenings of the jacket cell walls; opening by 1–4 longitudinal lines or irregularly; widely distributed throughout the world; approximately 30 genera and 550 species; sometimes the order Treubiales is separated from this order.

4. Order Jungermanniales

Leaves flattened, in 2 or 3 rows, usually broadened to attachment, often lobed; shoots reclining, erect, or pendent; rhizoids smooth-walled; archegonia terminating shoot, surrounded by a chlorophyllose sheath (perianth); sporophyte with seta; sporangium spherical to elongate, with elaters and thickenings of the jacket cell walls, opening by 4 longitudinal lines (rarely helical); distributed throughout the world, reaching greatest abundance in humid subtropical to temperate climates; contains at least 85 percent of the liverworts; conservatively, 300 genera and more than 7,000 species.

5. Order Sphaerocarpaceae

Essentially lobate thallus in all modern representatives; thallus of parenchyma cells reclining or erect, with smooth-walled rhizoids; each sex organ surrounded by an enveloping sac, lateral; sporangium spherical, lacking seta and elaters, opening by disintegration of the unornamented jacket cells; terrestrial except the aquatic genus *Riella*; distributed mainly in milder temperate climates; 3 genera with approximately 30 species.

6. Order Monocleales

Large thalli of mainly uniformly parenchymatous cells, reclining; thallus forked to irregularly branched; archegonia within a sleeve-like chamber behind the lobe apex; antheridia in pad-like receptacles in the same location on different thalli; sporangia elongate on a massive elongate seta, with long elaters and opening by a single longitudinal line; jacket with thickenings on cell walls; in South and Central America and New Zealand; a single genus, *Monoclea*, with 1 species.

7. Order Marchantiales

Thallus often of complex anatomy, with air pores on the dorsal surface, air chambers with chlorophyllose cells forming a photosynthetic area, and cells of the remainder of the thallus serving for storage; ventral scales often present; rhizoids; sex organs sometimes borne on a stalked receptacle; sporophytes with short seta or seta absent; sporangia spherical or elongate, opening by regular or irregular longitudinal lines, a cap-like lid, or decomposition; sporophytes often carried up from the thallus surface by elongation of the stalk of a receptacle, with the sporangia hanging downward; occupying a diversity of habitats—some can withstand extended periods of dryness while others are floating or submerged aquatics, and still others grow in humid shaded sites—approximately 27 genera and 450 species widely distributed throughout the world; the genus *Riccia* containing nearly half the species of the order.

Division Anthocerotophyta or Class Anthocerotopsida (or Anthocerotae; hornworts)

Protonema reduced to short filament or absent, differentiating the gametophore early after spore germination; rhizoids unicellular and smooth-walled; gametophore thallose, sometimes lobate; archegonium not a discrete structure, made up of an egg and neck canal cells embedded in the dorsal surface of the thallus; often several antheridia within a chamber embedded in the dorsal surface of the thallus; thallus sometimes with ventral pores, sometimes developing mucilage chambers; thallus lacking complex oil bodies; chloroplasts often solitary in each cell and often with pyrenoid; sporangium horn-shaped, usually with stomata in jacket; elaters often multicellular and often lacking helical thickenings; columella of sterile tissue extending the length of the sporangium, with the spore-bearing tissue overarched and sheathing it; sporangium indeterminate in growth from a basal meristem just above the foot; spores shed throughout the growing season by longitudinal lines of openings extending from the apex downward as the sporangium ages, sometimes (in *Notothylas*) by decomposition of the sporangium jacket.

1. Order Anthocerotales

Characteristics are those of the class; widely distributed in temperate to tropical latitudes, with greatest diversity in the tropics and subtropics; containing 6 or 7 genera and probably fewer than 300 species.

Division Bryophyta or Class Bryopsida (or Musci; mosses)

Protonema an extensive many-branched filament that precedes gametophore production; rhizoids multicellular, branched; gametophore leafy, with leaves spirally arranged, usually in more than 3 rows; gametophore usually not strongly flattened; sex organs usually with paraphyses among them; leaves unlobed and often with thickened midrib; cells usually lacking corner thickenings; oil bodies, if present, not complex; jacket of sporangium often with stomata; sporangium usually opening by apical cap (operculum); peristome teeth usually surrounding the sporangium mouth and influencing spore release; columella usually present, encircled or overarched by a spore-bearing layer; calyptra capping apex of elongating seta and influencing survival and differentiation of sporangium; spores generally shed over extended period; seta a rigid structure with internal conducting strand and holding sporangium well above gametophore in most instances.

1. Subclass Andreaeidae

Sporophytes usually lacking a seta; sporangium opening by longitudinal lines; sporangium with spore-bearing layer overarched and encircling the central columella; gametophore irregularly branched, dark-

pigmented, with spirally arranged leaves, attached to the substratum by rhizoids; leaves with or without midrib; paraphyses few or absent; sporophytes usually pushed beyond perichaetium on an elongate leafless extension of the gametophore (pseudopodium); mainly in cooler climates throughout the world, confined mainly to siliceous rock surfaces; 2 genera, *Andreaea* and *Andreaebryum*, with probably fewer than 100 species.

2. Subclass Sphagnidae

Sporophytes lacking a seta; subspherical sporangium opening by a lid (operculum) released explosively with the spores when sporangium dries, shrinks in diameter, and reaches high atmospheric pressure through compression of the gases within; protonema phase thalloid; branching in fascicles; leaf without midrib; leaf cells forming a network of elongate chlorophyllose cells surrounding dead swollen cells reinforced by fibril thickenings in walls and perforated by pores; sporophytes pushed beyond perichaetium by leafless extension of gametophore (pseudopodium); widely distributed in the world but forming extensive peatland mainly in boreal regions; 1 genus, *Sphagnum*, with more than 160 species.

3. Subclass Tetraphidae

Sporophytes with elongate seta; sporangium opening by an operculum exposing four multicellular peristome teeth that respond to moisture change to release spores gradually; spore layer forming a cylinder around central columella; protonema filamentous but with thallose flaps; gametophores erect, with rhizoids at base, leaves with midrib, all cells with chlorophyll; widely distributed in the Northern Hemisphere, with *Tetradontium* also present, but rare, in the Southern Hemisphere; 2 genera, *Tetraphis* and *Tetradontium*, with 3 or 5 species.

4. Subclass Polytrichidae

Sporophytes with elongate rigid seta containing conducting system; sporangium opening by operculum; numerous multicellular peristome teeth in a single concentric circle and overarched a membrane formed by the expanded apex of the columella (many rows of teeth and no membrane in *Dawsonia*); spores very small and released gradually through spaces between the teeth; spore layer forming a cylinder around central columella; gametophores erect, often with complex internal conducting system in stems and often leaves; leaves with numerous chlorophyllose elongate flaps on upper face; widely distributed throughout the world at most latitudes and altitudes, mainly terrestrial; approximately 16 genera and 370 species.

5. Subclass Buxbaumiidae

Sporophyte with elongate or short seta; sporangium asymmetrical, with operculum; peristome teeth sometimes in several concentric circles, the outer articulated, the inner forming a cone opened at the tip; spores released slowly when slight pressure on the sporangium surface causes the spores to puff out through the narrow mouth; gametophore sometimes extremely reduced and microscopic, always small but sometimes with leaves; widely but erratically distributed in temperate to tropical regions; 4 genera with approximately 40 species.

6. Subclass Bryidae

Sporophyte may have elongate seta, with or without conducting strand; sporangium diverse in form, with internal cylindrical columella encircled by spore-bearing layer, usually opening by operculum to expose articulated peristome teeth in 1 or 2 concentric circles; peristome teeth pulsating in response to moisture changes, extracting the spores from the sporangium and gradually releasing them; gametophores diverse in form and structure; widely distributed throughout the world in most habitats except the sea, representing more than 95 percent of the mosses; more than 650 genera and more than 9,000 species.

7. Subclass Archidiidae

Sporophyte with no seta; sporangia containing a restricted number of large spores (sometimes 4), lacking columella, opening by decomposition of the jacket; gametophore small, leaves with midrib; attached to substratum by rhizoids; of scattered distribution in temperate to subtropical climates; a single genus, *Archidium*, with approximately 26 species.

Critical appraisal

The order Takakiales is highly controversial; some researchers consider it sufficiently distinctive to separate it from the bryophytes to another phylum, while others merge it with the Calobryales. Discovery of sporophytes could settle the question. The order Anthocerotales is considered by some researchers to be so unrelated to bryophytes that it is placed in its own phylum, the Anthocerotophyta. The evolutionary lines of the class Bryopsida are most easily demonstrated by the subclasses. The treatment of orders and families remains in a state of flux, with widely varying opinions derived from differing interpretations of the taxonomic importance of characteristics. Even phylogenetic placement of the sequence of subclasses is difficult.

Fundamental classification of bryophytes is hampered by a lack of agreement concerning not only the critical features that define a bryophyte but also the criteria that can be used to interpret relationships. Consequently, there are considerable differences among classification systems. It is vital that there be an

adequate assessment concerning the diversity of bryophytes that now exist. The extremely limited number of researchers in the field of bryology greatly curtails the acquisition of this information.

Priyanka K. 8802387667

3. Alternation of Generations in the Bryophytes

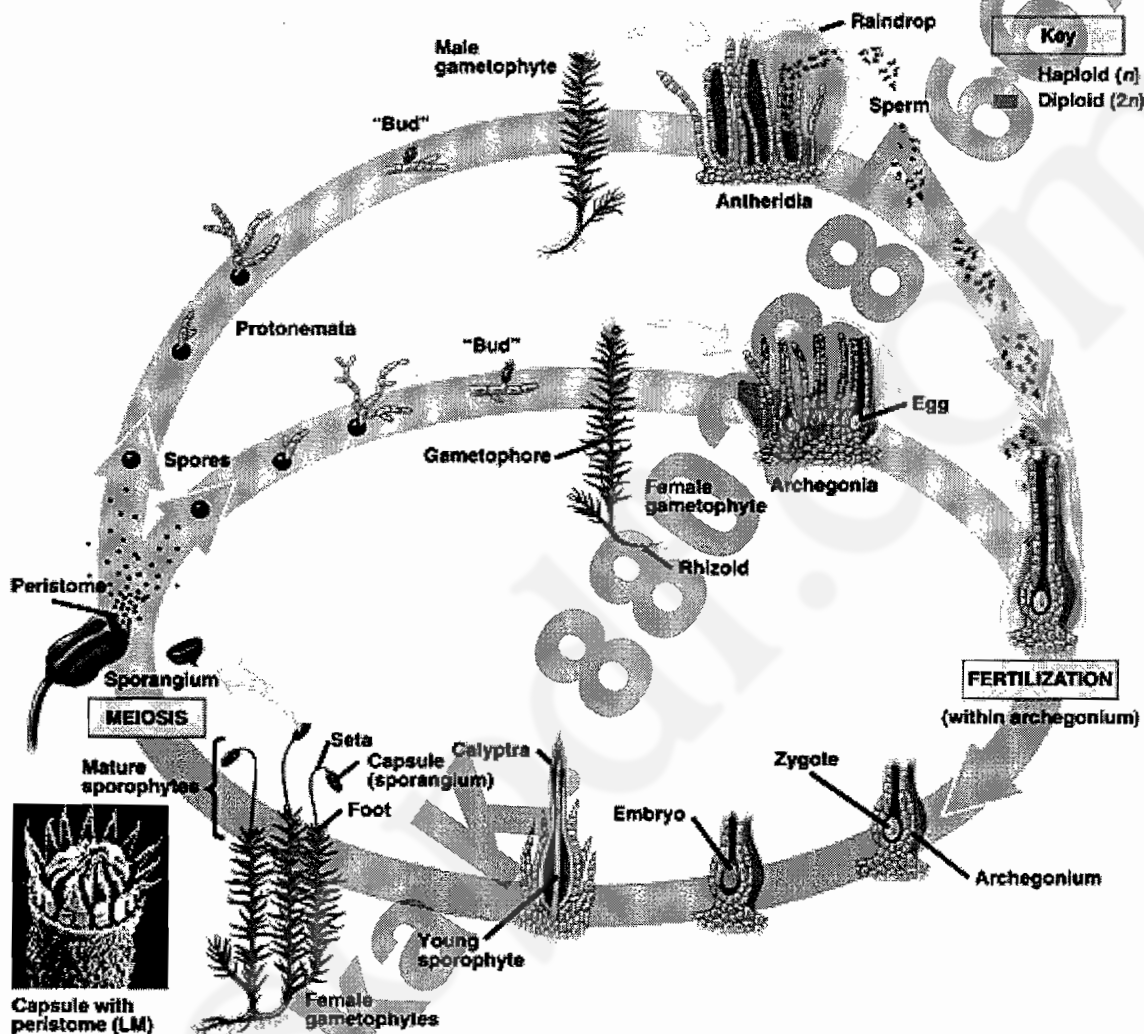


Figure 1: Life cycle of bryophytes

Introduction to alternation of generations

Alternation of generations, also called *Metagenesis*, or *Heterogenesis*, is a biological phenomenon of the alternation of a sexual phase and an asexual phase in the life cycle of an organism. The two phases, or generations, are cytogenetically and often morphologically distinct. The sexual phase, called the gametophyte, produces gametes, or sex cells; the asexual phase, or sporophyte, produces spores asexually. In terms of chromosomes, the gametophyte has a single (i.e., monoploid, or haploid) set, and the sporophyte has a double (diploid) set.

The life cycle of bryophytes is unique in always showing two cytogenetically and morphologically distinct phases, each represented by a separate adult. In simpler terms, the bryophytes always display the heteromorphic type of alternation of generations. In bryophytes, meiosis and fertilization divide the life of the plant into two distinct phases or "generations".

1. The gametophyte generation begins with a spore produced by meiosis. The spore is haploid, and all the cells derived from it (by mitosis) are also haploid. In due course, this multicellular structure produces gametes — by mitosis — and sexual reproduction then produces the diploid sporophyte generation.

2. The sporophyte generation thus starts with a zygote. Its cells contain the diploid number of chromosomes. Eventually, though, certain cells will undergo meiosis, forming spores and starting a new gametophyte generation.

Unique features of alternation of generations in bryophytes

Although alternation of generations occurs in the life cycles of algae, fungi, ferns, and seed plants the bryophyte alternation of generations is distinct in certain characters.

1. It is universally occurring among the bryophytes.
2. It is always heteromorphic.
3. The gametophyte is always the dominant generation – which means that it is nutritionally independent and ecologically more persistent than the sporophyte generation.
4. The sporophyte generation never becomes physically independent of the gametophyte stage. That is, the two alternating generations are always physically connected.
5. The sporophyte is not only physically connected to the gametophyte but it is nutritionally dependent on the gametophyte. Even when the sporophyte is photosynthetically capable, it never becomes self-sustainable in terms of nutrition.

The Figure 1 shows the pattern of alternation of generations in bryophytes using a moss life cycle as an example. Except for morphological details the scheme of alternation of generations in all the bryophytes is essentially the same.

The plan of alternation of generations in liverworts & hornworts

The plant bodies of liverworts and hornworts represent the gametophytic (sexual) phase of the life cycle, which is dominant in these plants. In the liverworts, the sporophyte is borne upon or within the gametophyte but is transitory. Liverwort and hornwort plants, depending on the species, may be bisexual or unisexual, and the sex organs may be distributed on the surface (*Riccia*, *Ricciocarpus*, *Sphaerocarpos*, *Pellia*) or localized in groups and borne on special branches (antheridiophores and archegoniophores) as in *Marchantia*. The sperms are biflagellate.

Release of the mature sperm and the process of fertilization require moisture in the form of heavy dew or raindrops. In all but a few genera (*Riccia*, *Ricciocarpus*), the developing sporophytes are actively photosynthetic—i.e., capable of utilizing light energy to form organic substances. They are, however, dependent on gametophytic tissues for water (and the inorganic salts dissolved in it) and probably derive and utilize in their nutrition some organic substances manufactured by the gametophytes. Liverwort spores are meiospores; i.e., they arise by meiosis from cells called sporocytes.

The sporophytes may consist almost completely of fertile (sporogenous) tissues (*Riccia*, *Oxymitra*), or they may contain sterile cells (nurse cells or elaters) among the developing spores. In *Marchantia* and *Porella*, a sterile foot and seta, or stalk, are present; the foot anchors the spore-bearing capsule (sporangium) to the gametophyte and also probably serves an absorptive function. The seta connects the foot and capsule. The elongation of the seta raises the capsule from its protective envelopes, thus, placing it in a favourable position for spore dispersal. The capsules of liverworts may shed their spores only by decay of the capsule wall and gametophytic tissues (*Riccia*, *Oxymitra*), or they may open irregularly or into two or four segments.

Spore germination in some species may occur immediately after deposition if the spores are in a favourable environment; or, as in other species, the spores may require a period of dormancy before germination.

The plan of alternation of generations in mosses

In mosses, as in liverworts and hornworts, the leafy shoots belong to the gametophytic phase and produce sex organs when they mature. The leafy shoots (often called gametophores, because they bear the sex organs) arise from a preliminary phase called the protonema, the direct product of spore germination. Filamentous, straplike, or membranous, it grows along the soil surface. A protonema of a moss may proliferate, apparently indefinitely, under favourable conditions and thus increase the population of leafy shoots that arise as buds. Under adverse conditions, certain buds and branches of the protonema may thicken their walls and thus serve to tide the species over an unfavourable growing period.

The antheridia and archegonia may be borne at the tips (apices) of the main shoots or on special, lateral branchlets. Both bisexual and unisexual leafy shoots occur, depending on the species. In a number of mosses (*Mnium*, *Polytrichum*, *Funaria*), the sexually mature shoots become recognizable through the production of special, prominent leaves that form an apical cup around the sex organs. If brightly coloured, the cup is often flowerlike. In species with bisexual leafy gametophores, the archegonia and antheridia may be present on the same apex (as can be seen, for example, in *Bryum*) or at the apices of separate branches as is exemplified in the moss *Funaria*.

The archegonia and antheridia of mosses are large enough in many species to be just barely visible to the unaided eye. The jacket cells of the antheridia are often coloured bright orange or rust; their sperm are biflagellate. As in liverworts and hornworts, rains and even heavy dews evoke the liberation of sperm and the opening of the mature archegonia so that fertilization may be accomplished.

The moss sporophyte, which is attached to the gametophyte, photosynthesizes during much of its development and is more or less self-supporting. It is, to a certain degree, dependent upon the gametophyte for nutrients such as water and mineral salts and, in some cases, even for elaborated foods.

After elongation of the moss sporophyte has ceased, the distal portion (farthest away) enlarges to form the capsule (sporangium), or spore-bearing region. The spores (meiospores), which arise by meiosis, are shed from the capsules gradually through a variety of mechanisms. After the operculum (cover) of the capsule has been shed, its mouth is usually partially closed by the peristome (teeth) and sometimes by associated structures. These teeth absorb moisture, and their resultant swelling and contraction open spaces through which the spores are shed.

Origin of alternation of generations

It is still a debated question. Two theories, namely, **antithetic** and **homologous** have been proposed to explain how alternation of generations originated.

Antithetic theory

It was the first to be proposed. On the basis of this theory the gametophyte or sexual plant represents the original generation. The sporophyte or the non-sexual organism is a new and different phase evolved by progressive elaboration of the diploid zygote of some algal ancestor like *Coleochaete*. It is interpolated in the life cycle of the gametophyte of primitive land plants between the two crucial points, **fertilization** and **meiosis** in response to a life in a drier environment. The factors, which caused its origin, are prompt germination of the zygote accompanied by delayed meiosis.

The result is the production of a small sporophyte of *Riccia* consisting simply of a spore case. With further elaboration and increased sterilization of the spore producing tissue a large sporophyte with differentiation into a foot, a seta, and a capsule is finally evolved.

Homologous theory

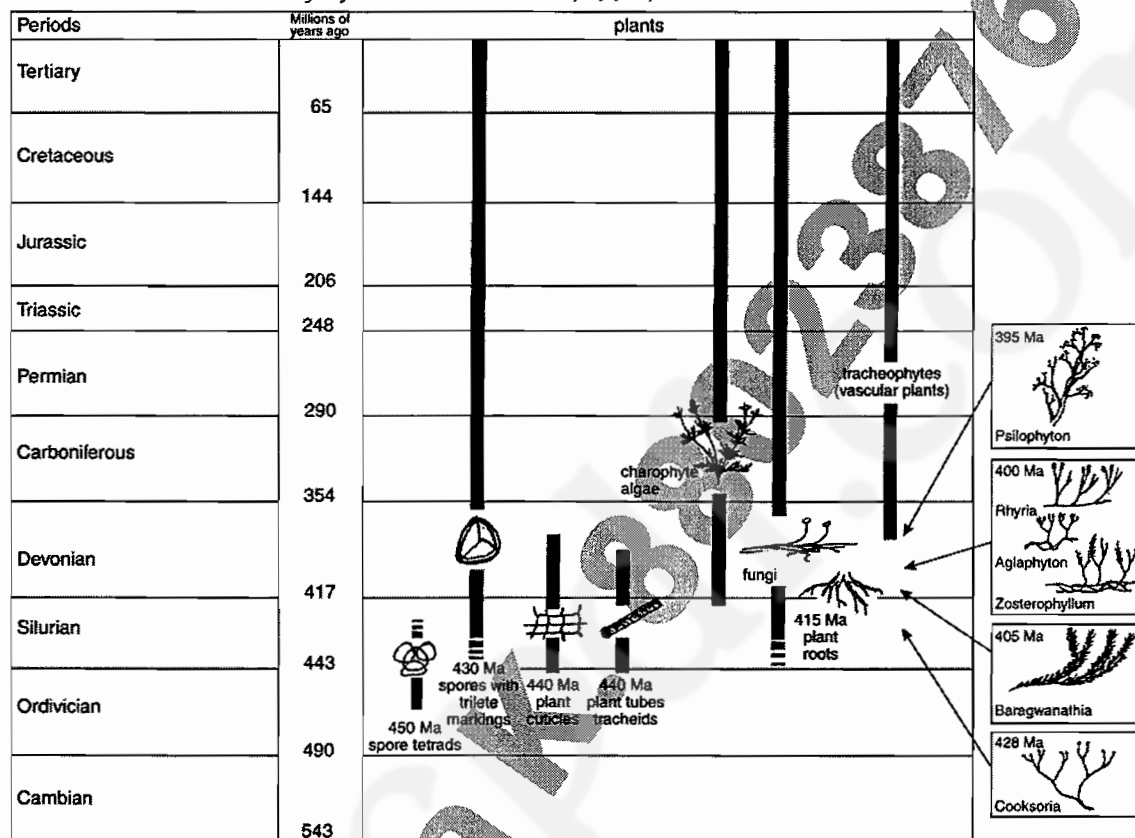
On the basis of this theory the sporophyte is simply a modification of the gametophyte and not a new generation.

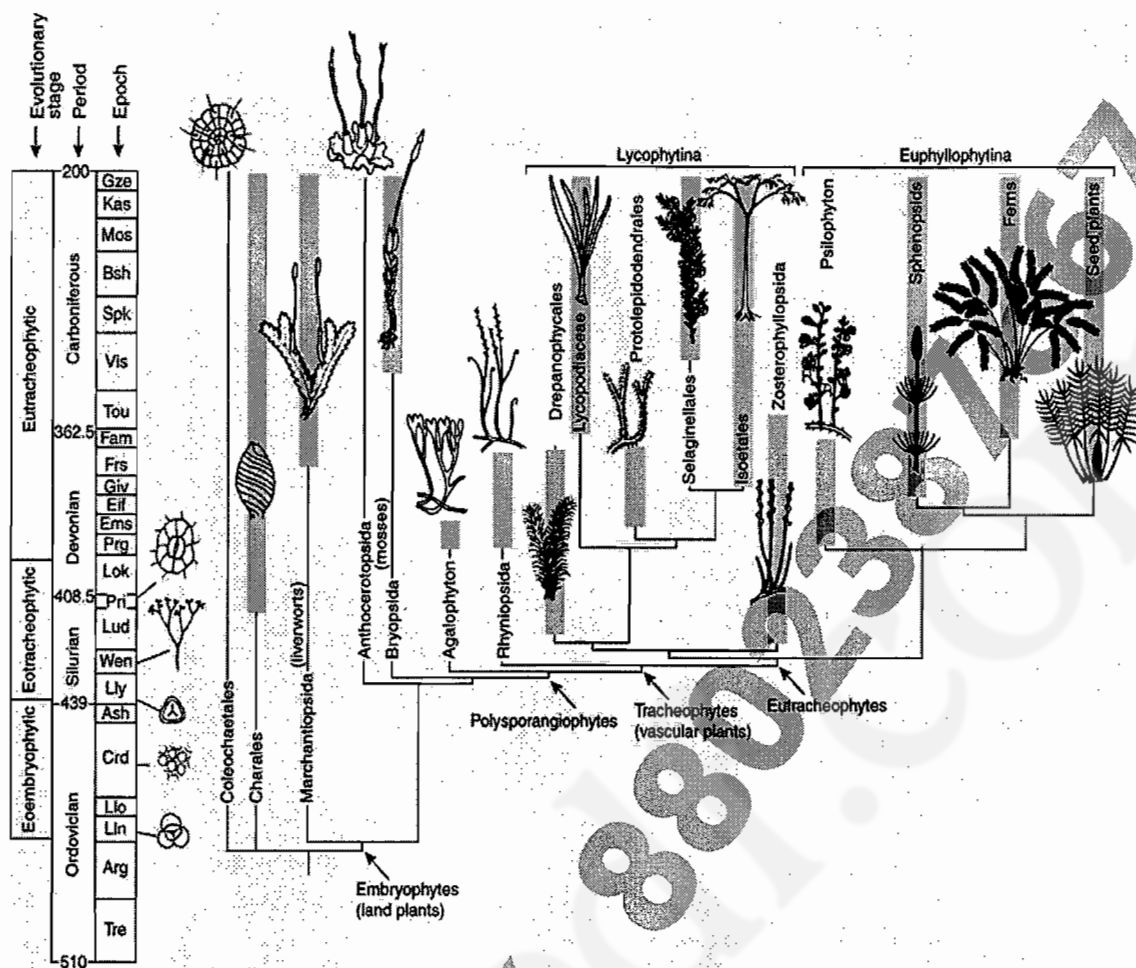
The advocates of this theory point to the fact that among the green algae the gametophyte plant reproduces by both the methods of reproduction. It bears spores and also the gametes. In the course of evolutionary sequence these two functions became separated in two distinct individuals. One of these produced the spores and the other produced gametes. The former came to be known as the **sporophyte** and the latter **gametophyte**. These two individuals occur regularly one after the other in the life cycle. The occurrence of two similar generations in most of the algae furnishes a strong argument in favour of this theory. The phenomenon of apospory and apogamy is another. Both the individuals in primitive land plants were photosynthetic and free living. Gradually, the sporophyte became attached to and partly parasitic on the gametophyte. Consequently, it became reduced.

4. Origin of the Bryophytes

Bryophytes are the most primitive plants, and their earliest somatic fossil record corresponds to *Hepaticities devonicus* of Devonian age, and consists of small fragments of an apparent hepatic thallus.

However, the sporopollenin coated fossilized tetrad organised spores unequivocally establish that the bryophytes originated from *Coleochaete* like ancestors about 445 million years ago late in the Ordovician. (Based on a detailed analysis of land plant relationships in a study titled *The Origin and Early Diversification of Land Plants: A Cladistic Study* by Kenrick and Crane, 1998.)





Ancestry

Features that bryophytes share with other green plants relate them to the green algae, and the green algae and bryophytes appear to share a common ancestor. The green algae show two evolutionary lines, the Chlorophyceae and Charophyceae (including Chaetophorales).

The bryophytes and other archegoniates (like Pteridophytes and Gymnosperms) have been derived from the charophycean line (including Chaetophorales). From the same charophycean line, the modern day Charophytes (including Chaetophorales) have also arisen. See the Figure on the next page.

This is the most widely agreed view on the origin of bryophytes that is also supported by molecular phylogenetic reconstructions — apart from many other lines of evidences.

The ecological factors

Land surfaces were available for colonization by plants from an early point in Earth's history. However, other essential environmental prerequisites were still developing. These included the formation of sizeable and stable near-shore environments, the formation of soils, and the development of suitable climatic and atmospheric conditions.

During the Cambrian and Ordovician (543–443 Ma) a combination of climate change and changing continental configurations resulted in widespread flooding of the continental plates. This was then followed by a period of glaciations at the end of the Ordovician (440 Ma) which led to a dramatic reduction in sea level and exposure of large areas of continental shelf.

Processes important for the early development of soils would have included atmospheric elemental input, and weathering by acid rain and organic acids produced by early microbial organisms and lichens. Geological evidence indicates that by the end of the Ordovician (~440 Ma) well-established soil profiles had developed.

By the late Ordovician (458–443 Ma) global climates were becoming much more variable and certain regions were becoming cool and moist. These climatic changes are attributed to two main factors: a gradual

reduction in atmospheric CO₂, leading to a reduced greenhouse effect, and the formation of glaciers at the South Pole.

It was under these conditions that the bryophytes perhaps arose from a Chaetophoralean plexus. The acquisition of a cuticle, gametangia, a protected embryo, protective pigments, thick spore walls with a protective polymer, and a mutualistic association with a fungus are all defining characters of plants, and all are associated with the adaptation of plants to life on land.

The three groups of the bryophytes have arisen separately but from a common large ancestral stock. The liverworts were the first to arise, then the hornworts and finally the mosses. A simplified picture of bryophytic origin and phylogenetic relation with other groups is given below (from *The Origin and Early Diversification of Land Plants: A Cladistic Study* by Kenrick and Crane, 1998).

The evidences

1. In general, as in vascular and non-vascular land plants, all green algae contain chlorophyll *a* and *b*, true starch, and cellulose in their cell walls. However, particular groups of green algae, such as the Charophyceae have an even closer biochemical affinity.
2. Most of the metabolic processes are common between the charophytes and the modern day bryophytes.
3. A study of the enzymes involved in the non-mitochondrial part of respiration in groups of extant green algae and land plants has demonstrated that, in the Charophyceae, the enzyme glycolate oxidase, characteristic of land plants, replaces glycolate dehydrogenase which is present in other groups of algae. Both enzymes are important as scavengers of carbon that would otherwise be lost by excretion from photosynthetic cells. However, these enzymes differ in cellular location and in the process of retrieving carbon. It has been demonstrated that the presence of glycolate oxidase would have been particularly advantageous to early land dwelling plants.
4. Another enzyme present in the Charophycean algae and land plants but not in other green algae, is an enzyme called copper zinc superoxide dismutase that eliminates damaging oxygen radicals from plant cells. The advantages of this enzyme to land dwelling is in giving protection from ROS induced damages.
5. The presence of sporopollenin in spore walls and cuticles in the early fossil bryophytes and certain Charophycean green algae, for example, *Spirogyra*, *Chara*, and *Coleochaete* is another evidence of charophycean ancestry of the land plants.
6. In both charophytes and the modern day bryophytes the cellulose synthesizing enzyme complex is typically rosette shaped.
7. The examination of group II introns (non-coding DNA that interrupts short sections of coding DNA) found in the tRNA genes of all land-plant chloroplast shows remarkable similarity between charophytes and the modern day bryophytes.
8. Complete nuclear-encoded small-subunit 18S rRNA (= SSU rRNA) gene sequences were determined by Kranz HD, Miks D *et al* in 1995 for the prasinophyte green alga *Mantoniella squamata*; the charophycean green algae *Chara foetida*, *Coleochaete scutata*, *Klebsormidium flaccidum*, the bryophytes *Marchantia polymorpha*, *Fossombronia pusilla*, and *Funaria hygrometrica*; and the lycopod *Selaginella galleottii* to get a better insight into the sequential evolution from green algae to land plants. Based on this analysis the evolutionary botanists agree that the bryophytes originated from *Coleochaete* like ancestors about 450 million years ago.
9. In common with the green land plants, the charophycean line

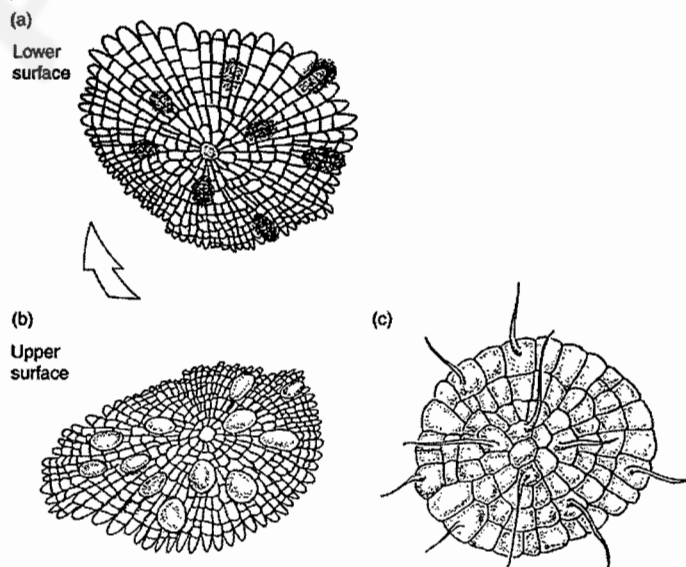


Figure 2: Left: *Parkia* – Right: *Coleochaete*

- possesses the same type of cell division with persistent spindles. It may form phragmoplasts. The chlorophycean line, to which most green algae belong, lacks persistent spindles, forms phycoplasts.
- The Morphology of the sperm cell is remarkably similar between the charophytes and the modern day bryophytes.
 - Since the ferns and their allies possess gametophytes roughly similar to those of some bryophytes and since the sex organs are also structurally similar, these plants appear to have shared a common origin with the bryophytes.
 - The hornworts carry pyrenoids in the plastids, a hallmark feature of the green algae.
 - The fossil bryophyte *Parka* and modern day algal genus *Coleochaete* show remarkable morphological similarities. (Figure 2: Left: *Parka*— Right: *Coleochaete*).
 - There is a remarkable similarity in the life cycles as well (shown in the Figure on the next page).

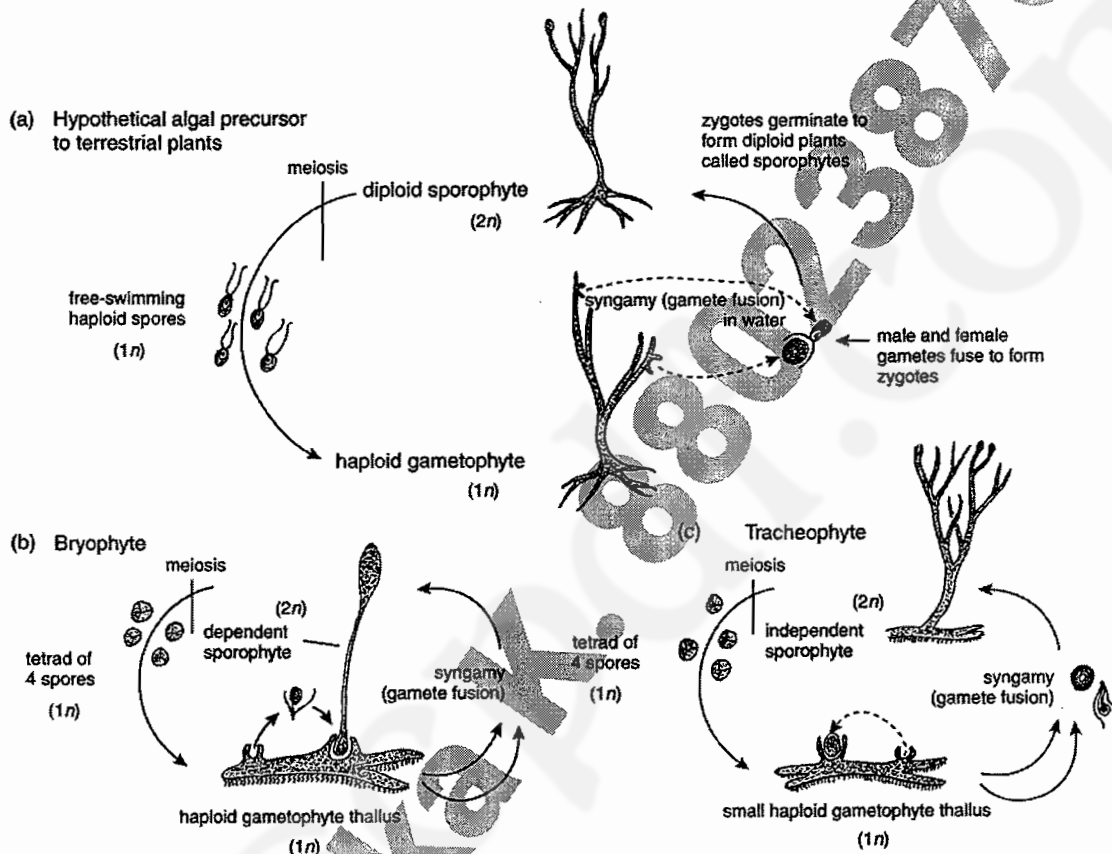


Figure: Evolution of Bryophyte Life Cycle

5. Land Adaptations of the Bryophytes

Adaptations for land habitat

Adaptation to land environment or sub-aerial life involved the development of certain features that were not possessed by their aquatic ancestors. The acquisition of a cuticle, gametangia, a protected embryo, protective pigments, thick spore walls with a protective polymer, and a mutualistic association with a fungus are all defining characters of plants, and all are associated with the adaptation of plants to life on land.

1. **Development of organs for attachment and absorption of water:** Unlike algae the bryophytes which grow on land are not bathed in water. They must absorb it from the soil and be attached to it for support. For this purpose the bryophytes develop special, hair-like structures called the **rhizoids**. Like root hairs the rhizoids function as absorbing and attaching organs.
2. **Protection against desiccation:** The thick, compact multicellular, thallus-like plant body covered with an **epidermis** is protected to a certain extent against the drying effects of air. Of the numerous cells constituting it only the epidermal cells, which form only a small percentage of its total surface, are directly exposed to the dry air. Further even the free surface of the epidermal cells, in some species of liverworts, is coated with a waxy substance like **cutin** that is waterproof and thus reduces the rate of water loss. Moreover, **total surface area of a compact body is reduced** in proportion to its volume.
3. **Absorption of carbon dioxide from the atmosphere for photosynthesis:** In many liverworts there are numerous pores on the upper surface of the thallus. These are called the **air pores**. They facilitate gaseous exchange between the atmospheric air and the interior of the thallus.
4. **Protection of reproductive cells from drying and mechanical injury:** The sex organs in the bryophytes are multicellular and jacketed. The **jacket of sterile cells** around the sperms and eggs is an adaptation to a life on land. It protects the sex cells against the drying effects of air.
5. **The fertilized egg is retained within the archegonium.** Here it obtains food and water from the parent plant and is protected from drying as it develops into an **embryo**. This adaptation is essential for the survival of the land plants. It ensures nursing of the young embryo and its protection against mechanical injury.
6. The **thick-walled, wind-disseminated spores** and the primitive vascular system in the form of a conduction strand are the other adaptations to land habit.
7. A well established **heterotrichous body** ensures a better division of labour among the bryophytes. Many bryophytes use one part of their body for assimilatory and storage purposes and another for reproductive purposes.
8. Most of the bryophytes have **phyllids (leaves)**, which a flattened photosynthetic organs. The flattened structure **increases the surface area available for photosynthesis**.
9. The bryophytes also developed **bitter chemicals** to avoid predation.
10. The bryophytes also developed **several modes of vegetative reproduction**, which enables the species to survive even if sexual reproduction fails.
11. The bryophytes also have **great power of regeneration**, to minimize the effect of predation etc.
12. The bryophytes also developed **several hygroscopic modes of spore dispersal** under appropriately dry conditions, which maximizes the reproductive success.

Limitations of adaptations for land habitat

1. The bryophytes, however, cannot carry on their reproductive activities without sufficient moisture. Presence of water is necessary for sexual reproduction. Without it the sex organs do not reach maturity and do not dehisce. Water is essential for the transfer of sperms to the archegonia. The retention of swimming sperms is an algal characteristic. The bryophytes thus rely on water for the act of fertilization.
2. They have inefficient absorbing organs in the form of rhizoids. Consequently they are unable to grow during dry periods. They require sufficient moisture both for reproduction and successful vegetative growth. This explains why the bryophytes usually inhabit moist, shaded situations (amphibious habitat) or grow in places where water is abundant at least at some season. Since the bryophytes

usually grow in amphibious situations and cannot complete their life cycle without external water they can very appropriately be called the **amphibians** of the plant world.

3. **True stomata are mostly lacking** in the gametophytic generation which is the dominant generation. In the absence of true stomata, the CO₂ acquisition for photosynthesis is hydro-economically expensive.
4. The bryophytes are **totally devoid of true vascular system**, due to which:
 - i. The plant is **poikilohydric**
 - ii. A **poor water conduction** occurs
 - iii. A **poor translocation of nutrients** results from one part of the plant to another
 - iv. The plant **can not gain much height** — due to which • it can not capture much insolation and • can not avoid much predation (An application of Ryan and Yodder's Hydraulic Limitation Theory, 1993).
5. The bryophytes are **totally devoid of lignified mechanical tissue system**, due to which the plant **can not gain much height** and can not capture much insolation plus can not avoid much predation.
6. **The dominant phase of the life cycle is Haploid** — due to which the plant is more vulnerable to lethal mutations.

It was due to these structural, physiological and genetic limitations that the bryophytes could never form the dominant flora on the land despite being the pioneer colonizers of the terrestrial habitat.

6. Liverworts

Introduction

Liverworts get their name from their shape. In medieval times, people thought that the shape of a plant would tell about the part of the body it could help cure. Thalloid liverworts look like the liver. Like moss, liverworts grow in moist habitats.

Liverworts are like mosses in the fundamental features of their life cycle, but differ greatly in organization of their mature gametophytes and sporophytes. Liverwort gametophytes can be either leafy shoots or flattened thalli. In the leafy forms, the leaves [strictly speaking the phyllids] are arranged on the stem in one ventral and two lateral rows or ranks, rather than in spirals like the mosses. The leaves are mostly one cell layer thick throughout, never have a midvein and are usually divided into two or more parts called lobes. The ventral leaves, which actually lie against the substrate, are usually much smaller than the lateral leaves and are hidden by the stem. Anchoring rhizoids, which arise near the ventral leaves, are colorless and unicellular. The flattened ribbon-like to leaf-like thallus of the thallose liverworts can be either simple or structurally differentiated into a system of dorsal air chambers and ventral storage tissues. In the latter type, the dorsal epidermis of the thallus is punctuated with scattered pores that open into the air chambers. Liverworts synthesize a vast array of volatile oils, which they store in unique organelles called oil bodies. These compounds impart an often spicy aroma to the plants and seem to discourage animals from feeding on them. Many of these compounds have potential as antimicrobial or anticancer pharmaceuticals.

Liverwort sporophytes develop completely enclosed within gametophyte tissues until their capsules are ready to open. The seta, which is initially very short, consists of small, thin-walled, hyaline cells. Just prior to capsule opening, the seta cells lengthen, thereby increasing the length of the seta upto 20 times its original dimensions. This rapid elongation pushes the darkly pigmented capsule and upper part of the whitish seta out of the gametophytic tissues. With drying, the capsule opens by splitting into four segments, or valves. The spores are dispersed into the winds by the twisting motions of numerous intermixed sterile cells, called elaters. In contrast to mosses, which disperse their spores over several days, liverworts disperse the entire spore mass of a single capsule in just a few minutes.

Characteristic features

1. The plant body thalloid or foliose.
2. Rhizoids are present and without septa; usually simple and tuberculate; ventral scales present.
3. Cells have chloroplast without pyrenoids.
4. Green cells contain simple or compound oil bodies
5. Sex organs develop from superficial cells on the dorsal side of the thallus except when they are terminal in position.
6. Sporophyte simple or differentiated into foot, seta and capsule.
7. Columella is absent, elaters maybe present.
8. Sporophyte completely dependent upon the sporophyte for nutrition.
9. Dehiscence of sporogonium is irregular or regular.
10. Sporogenous cells develop from endothecium.

Taxonomy

Please refer to the earlier section on Systematics of Bryophytes.

Gametophyte, sexual reproduction and sporophyte

The dominant plant in the liverworts, like all other bryophytes, is the gametophyte. For a number of reasons, we can call it the most primitive form of land plant – a fact that is also supported by molecular phylogenetic studies [a study titled *The Origin and Early Diversification of Land Plants: A Cladistic Study*, Kenrick and Crane (1998)].

It is a dorsiventral thallus-like structure, with or without phyllids, which remains small in size. It lacks the vascular tissue, which is essential for conduction of water and minerals. It has no supporting or mechanical tissue. Besides, the absorbing organs in the form of rhizoids – not as efficient as the roots. Secondary meristems are absent. Consequently, a vegetative plant body remains a thin-walled, porous, flat, thallus-like structure, growing prostrate and close to the surface of the substratum. The corticating-

tissues, which conserve water are lacking, hence the prostrate habit is useful. It reduces the exposed surface and the major portion of the plant body remains in direct contact with soil moisture. Besides, it furnishes maximum surface for fixation and absorption of water.

The gametophyte of liverworts is green and independent. We find two basic types of gametophyte constructions among the liverworts.

1. Some liverworts like advanced Metzgeriales, Marchantiales, Sphaerocarpaceae and Monocleales have a thalloid plant body. It is flat and conspicuously lobed or branched. The branching is dichotomous..
2. There are liverworts like Takakiales, Calobryales, Jungermanniales and primitive Metzgeriales in which the gametophyte plant is leafy. It consists of a central branched axis (stem) bearing leaf-like green expansions. The leaves are arranged in three rows; two rows in lateral sides and the third row on ventral side of axis. The leaves of third row in prostrate form of leafy liverwort are called amphigastria or under leaves. The under leaves gradually reduce in size and ultimately disappear. In *Fossombronia* only two rows of lateral leaves are present.

Both the so-called leaves and the stem lack vascular tissues. The leafy or foliose liverworts are more numerous. Both the thalloid and the leafy gametophytes are anchored to the substratum by unicellular unbranched rhizoids.

It is believed that the thalloid form might have evolved from leafy form of liverworts. *Schiffneria*, a member of order Metzgeriales represents a transitional stage in between thalloid and leafy forms. In this genus, the vegetative plant body is thalloid but at reproductive stage the short abbreviated leafy shoots bearing sex organs are produced from the ventral surface of thallus.

As the usual method of reproduction in the bryophytes, it consists in the union of gametes and subsequent production of meiospores.

Distribution of the sex organs: The male and the female sex organs in some species of liverworts are borne on different plants. The examples of this kind are *Riccardia indica*, *Pellia calycina* and *Marchantia*. Such species are called **dioecious**.

There are others in which two kinds of sex organs are developed on the same plant. They are called **monoecious** e.g. *Riccardia multifida*, *Asterella blumeana* and *Pellia epiphylla*.

Location of sexual development: The sex organs may be borne dorsally in thalloid liverworts or at the anterior end of the leafy liverworts. They can be embedded in the tissue of the thallus (*Riccia*) or raised on special upright branches (*Marchantia*) called the **gametophores**. In leafy liverworts, the sex organs are never borne upon stalked receptacles. In Jungermanniales, which is the major order of leafy liverworts, the antheridia are enclosed within sac like male bracts and archegonia within perianth. The perianth is innermost covering around the archegonia.

Process: In liverworts the sperms are biflagellate. They are produced two per androcyte or sperm mother cell by a process of mitosis. Release of the mature sperm and the process of fertilization require moisture in the form of heavy dew or raindrops. Only a single sperm cell can fertilise a given archegonium since it contains only a single egg cell.

The zygote resulting from fertilization begins to divide and forms the embryo that draws nutrition from the parent gametophyte through the placental tissue. Eventually, the embryo divides and differentiates into the sporophyte.

It receives a special name **sporogonium** in the bryophytes. The sporogonium is without differentiation. Consequently, it suffers from certain disabilities. It lacks direct contact with the soil as it has no roots or other basal appendages of any kind. It remains attached by the foot to the parent gametophyte (thallus) which provides for its nutrition from its own resources. Owing to the limited ability of the parent gametophyte plant for absorption and conduction, the possibilities of the development of the parasitic sporogonium in the liverworts are limited. Moreover, it has no stem to bear leaves or to elevate and support photosynthetic appendages. Naturally it is doomed to remain small. The other disabilities it suffers from are :

1. Absence of meristematic tissue.
2. Absence of any kind of lateral appendages and of branching habit.
3. Continuity of the archesporium leading to simultaneous ripening of spores.

All these factors collectively account for the liverwort sporogonium not progressing beyond a limited size. It remains dwarfed.

The sporophytes may consist almost completely of fertile (sporogenous) tissues (*Riccia*, *Oxymitra*), or they may contain sterile cells (nurse cells or elaters) among the developing spores.

In all but a few genera (*Riccia*, *Ricciocarpus*), the developing sporophytes are actively photosynthetic—i.e., capable of utilizing light energy to form organic substances. They are, however, dependent on

gametophytic tissues for water (and the inorganic salts dissolved in it) and probably derive and utilize in their nutrition some organic substances manufactured by the gametophytes.

In some species the sporogonium is differentiated into three parts, a foot, a **seta** and a **capsule**. In a few others (*Riccia*) both the foot and seta are absent. The sporogonium of *Corsinia* consists of the foot and the capsule. The seta is lacking. The Jungermanniales have the ovoid or spherical capsule elevated on a long, fragile seta. The sporogonium is a specialized body which is solely devoted to the production of meiospores and their dispersal.

A large proportion of the cells of the endothecium are devoted to spore formation as compared with the other two classes of the bryophytes. The endothecium cells which do not form spores remain sterile and develop into elaters. In some genera of the Metzgeriales, a tuft of fixed elaters occurs at the base of the capsule (*Pellia*,) or at the apex (*Riccardia*) – this elateral cluster is usually called the **elaterophore**.

Sporogenesis . It is a process whereby the diploid protoplast of the spore mother cell (sporocyte) is transformed into haploid dispersal units with resistant walls termed the *spores* or more appropriately the *meiospores*. The process is complex and comprises an integrated series of events involving a special kind of nuclear division termed **meiosis**.

At the onset of sporogenesis, the spore mother cells increase in size and their walls thicken. The diploid nucleus of the spore mother cell (sporocyte) undergoes two successive division. The cell walls between the resultant 4 haploid nuclei are laid simultaneously after the second division dividing the spherical spore mother cell into four equal cells arranged in a tetrahedral manner. These cells thicken their walls and ripen into spores. The four spores of each sporocyte remains together until they are fully grown. It is called a **spore tetrad**. In a ripe spore two, sometimes three distinct layers can be differentiated in the spore wall. The outer layer is called the **exosporium** (exine or exopore) the inner one **endosporium** (intine) and the median one **mesosporium**.

The liberated meiospore consists of a tiny mass of protoplasm surrounded by a protective, stratified spore wall or coat technically known as the *sporoderm*, which is differentiated into two or three layers, namely, exospore, the mesospore and the endospore. The reserve food is stored in the form of starch and fatty oil droplets. Besides, there are granules mostly albuminous in nature. The small haploid nucleus is usually located in the center.

The spores of Marchantiales and Metzgeriales are comparatively larger and highly ornate whereas those of Jungermanniales are small, spherical and with relatively simple ornamentation. Furthermore the spores of Jungermanniales are apolar-one with no triradial mark whereas those of Marchantiales are strongly whereas those of the Jungermannie become deeply 4-lobed in the meiotic prophase.

On germination each spore produces the alternate plant in the cycle. It is the thallus (gametophyte) and not the sporogonium (sporophyte). Meiospore thus is the initial cell of the free living gametophyte in the sexual life cycle of the liverworts.

The Jungermannioid group

Traditionally, liverworts have been subdivided into two groups.

1. **The Marchantioid group**, or complex thalloids. Crandall-Stotler & Stotler (2000) recognized the group as a class, Marchantiopsida.
2. **The Jungermannioid group**: Crandall-Stotler & Stotler (2000) recognized the group as class Jungermanniopsida. It comprises two morphological subgroups.
 - a. The anacrogynous, simple thalloids. This group is recognized as subclass Metzgeriidae. Their diagnostic characters are: Thallöse, with the thallus mainly of uniformly thickened cell walls, usually reclining but sometimes erect; branching varies from forked to regularly pinnate or irregular; smooth rhizoids on the undersurface; sex organs lateral; sporophytes with elongate seta; sporangia spherical to elongate, with elaters and thickenings of the jacket cell walls; opening by 1–4 longitudinal lines or irregularly.
 - b. The acrogynous, leafy hepatics. This group is recognized as subclass Jungermanniidae. Their diagnostic characters are: Leaves flattened, in 2 or 3 rows, usually broadened to attachment, often lobed; shoots reclining, erect, or pendent; rhizoids smooth-walled; archegonia terminating shoot, surrounded by a chlorophyllose sheath (perianth); sporophyte with seta; sporangium spherical to elongate, with elaters and thickenings of the jacket cell walls, opening by 4 longitudinal lines (rarely helical); distributed throughout the world, reaching greatest abundance in humid subtropical to temperate climates; contains at least 85 percent of the liverworts.

In older classifications (Parihar, 1958), the Jungermannioid group was treated at the level of an order, Jungermanniales under the class Hepaticopsida. In the current thinking, the the Jungermannioid group has the rank of a class Jungermanniopsida.

Jungermannioptida with about 244 genera and 9000 species are the largest liverwort group. They are represented in India by genera such as *Pellia*, *Riccardia*, *Porella*, *Diplophyllum*, *Fossombronia*, *Cephalozia*, *Sewardiella*, *Solenostoma*, *Plagiochila*, *Radula*, *Lophozia*, *Jungermannia*, *Riella*, *Aneura*, *Mastigobryum*, and *Metzgeria*.

Salient Features of Jungermannioptida

1. The gametophytic plant body is thalloid or foliose. The thalloid forms are simple, dorsiventral and dichomotously branched (e.g. – *Pellia*, *Sewardiella*, *Aneura*), whereas the foliose forms (also known as leafy liverworts) are differentiated into axis and leaves (e.g. – *Porella*, *Frullania*, *Radula*, *Diplophyllum*). Besides, some members show a gradual transition from thalloid to leafless habit (eg. – *Petalophyllum rolfsii*).
2. The thallus does not show differentiation into photosynthetic and storage regions as in Marchantiopsida. Usually, all the cells of the thallus have chloroplasts.
3. Air pores and air chambers are altogether absent.
4. The thallus remains attached to the substratum with the help of smooth walled rhizoids.
5. Tuberculate rhizoids and scales are absent.
6. The thallus grows by a single apical cell.
7. Vegetative propagation takes place usually by means of gemmae but some members (e.g. – *Calycularia*, *Leptolejeunia*) multiply with the help of adventitious branches and leafy propagules.
8. Antheridia are globose and borne on long stalks.
9. The neck and venter of the archegonium are almost of the same diameter.
10. The neck is made up of 5 vertical rows of neck cells.
11. The sporophyte is differentiated into foot, seta and capsule.
12. The capsule jacket is multilayered; at maturity it splits into 4 regular valves.
13. The archesporium develops from the endothecium.
14. Spore mother cells become deeply four lobed prior to sporogenesis.
15. In some members an elaterophore is present in the basal (eg – *Pellia*) or apical (eg – *Riccardia*) region of the capsule.

Current view on Jungermannioptida

Recent molecular phylogenetic studies (e.g. Heinrichs et al. 2005, 2007, Forrest et al. 2006) have greatly modified this morphology based concept.

These studies show that the simple thalloids are paraphyletic with representatives in four of the six backbone clades.

One of these, comprising the Haplomitriaceae and Treubiaceae, has been identified as the earliest diverging lineage of the hepatics and relegated to a third class, Haplomitriopsida (Forrest et al. 2006).

7. Genus: Marchantia

Family : Marchantiaceae

1. The dorsal surface of the thallus is marked by rhomboidal areas (areolae), each with a central pore.
2. Internally the thallus is chambered, hence also called chambered hepatic.
3. The sporogonium differentiated into foot, seta capsule, elaters present e.g., *Marchantia*.

Genus : Marchantia

Marchantia is a cosmopolitan genus with about 65 species (named after N. Marchant of France), occurring in **dense green patches** on the **moist cold and shady terrestrial habitats**. In India *Marchantia* mostly occurs in the hills on forest floor, moist and shaded cliffs, tree trunks or the muddy banks of water courses. A few species like *M. polymorpha*, and *M. palmata* are present in the plains also. A variety of *M. polymorpha* (var. *aquatica*) is submerged aquatic.

Morphology

The gametophyte plant body is a green **prostrate dorsiventral dichotomously branched thallus** about 2-10 centimetres in length and 0.5-3 cm in breadth. The lobes may be narrow and linear (e.g., *M. nepalensis*) and or broad (e.g., *M. palmata*) with a smooth or wavy margin. They possess a broad dark and median thickening called **midrib**. It is marked by a shallow **groove** on the dorsal side and a low ridge on the ventral side. Each lobe has an **apical notch** which contains the growing point.

The upper or dorsal surface of the thallus is dark green while the ventral surface is pale green. The upper surface is divided up into rhombial or polygonal areas called **areoles** or **areolae**. These areas indicate the presence of underlying air chambers. In the centre of each areole lies a slightly, raised **ventilating air pore**, which is narrower in the apical region as compared to that of the posterior end.

In certain seasons small cup-like structures, the **gemma cups**, are formed dorsally along the midrib. The gemma cup bears **gemmae** which help in vegetative multiplication.

In the adult condition certain upright branches, known as **gametophores** bearing sex organs arise from notches of the thallus.

From the **ventral surface** of the thallus arise numerous **rhizoids** and **scales**. The rhizoids are colourless or pale brown, unicellular and unbranched tubular outgrowths. They contain very little of protoplasm. The rhizoids are of two types, **smooth-walled** (simple) and **tuberculate**. The smooth walled rhizoids are more concentrated near the midrib. They grow directly downwards and penetrate the soil and help in fixation and absorption of water and mineral salts from the soil. The tuberculate rhizoids commonly arise from the sides near the scale bases and converge towards the midrib. They are narrow and have comparatively thick walls. From their inner walls develop numerous peg-like outgrowths or tubercles which project into the lumen of the rhizoids without forming complete partitions. The tuberculate or peg rhizoids do not penetrate the soil but instead run horizontally along the underside of the thallus to form a capillary conducting system.

The **scales** or **amphigastria** are purple or violet coloured plate-like outgrowths of the thallus. Each scale consists of a plate of somewhat thick walled cells. It may be 2-3 celled thick at the base. There may be 2 (e.g., *M. geminata*) or 3 (e.g., *M. polymorpha*) rows of scales on either side of midrib. The three rows are respectively known as **median**, **laminar** and **marginal**. The median scales are also called **appendiculate**. They are large and wedge shaped in outline. Each median scale is differentiated into three parts—body, neck and appendage. The body has an extended or decurrent base. Neck is like constriction.

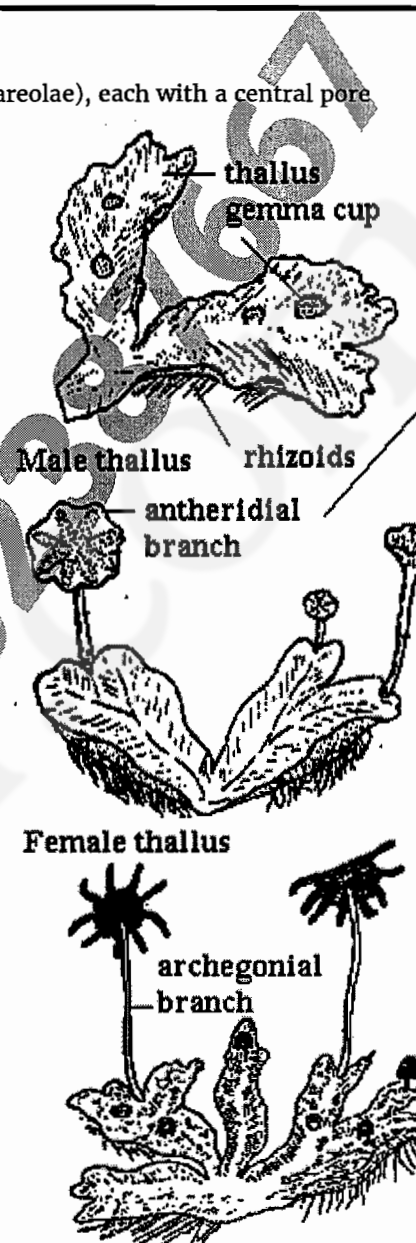


Figure 1: *Marchantia* gametophyte

The outer scales (marginal and laminar) are simple and they do not possess an appendage.

The cells of the scale contain anthocyanin pigment. Here and there the scales have oil cells. The margin often bears few celled mucilage papillae. The scales are crowded over the growing point. They protect it from mechanical injury. Scales retain and transport water by capillarity. Their mucilage protects the thallus and the growing point from desiccation.

Growth of the gametophyte: Growth occurs by a row of apical initial cells lying in the apical notch. By the maturation of some of the middle cells, two growing points are formed in dichotomous branching.

Internal Structure

The gametothallus of *Marchantia* is several layered thick in the middle and few layered near the margins. It is divisible into two regions upper photosynthetic and lower storage.

1. Photosynthetic Region.

- Dorsal green portion of the thallus
- Consists of two parts—**upper epidermis** and **air chambers**.
- Upper epidermis is cutinized; single layered and covers the dorsal surface of the thallus. The epidermis is punctured by many barrel-shaped **air pores**. They allow the exchange of gases.
- Each pore is surrounded by 16–40 cells having cutinised walls and few chloroplasts. These cells are arranged in 4–8 superposed ringed tiers. Each tier consists of 4–5 cells. The cells of the uppermost and lowermost tiers form circles of the smallest diameter. The pores of *Marchantia are not true stomata.*
- Each **air chamber** communicates with the exterior through an air pore in the upper epidermis. The floor of the air chambers is made of green cells similar in shape to those of the upper epidermis. The air chambers are separated from one another by single layered partition walls which are 2–4 cells in the height. The cells are large and contain abundant chloroplasts. From the floor of each air chamber arise many short simple or branched **assimilatory** filaments of green oval cells containing many discoid chloroplasts.

2. Storage Region

- Lower non green region of the thallus which lies below the photosynthetic region.
- Several cells thick in the midrib region and gradually thins out towards the margins.
- Consists of compactly arranged parenchymatous cells rich in starch and proteins.
- A few individual cells may contain a large oil body (oil cells) or mucilage (mucilage cells).
- Covered on the ventral side by the lower epidermis.
- Certain cells of the lower epidermis give tubular outgrowths in the form of the smooth-walled and tuberculate **rhizoids**. The scales or **amphigastria** also arise from the lower surface.

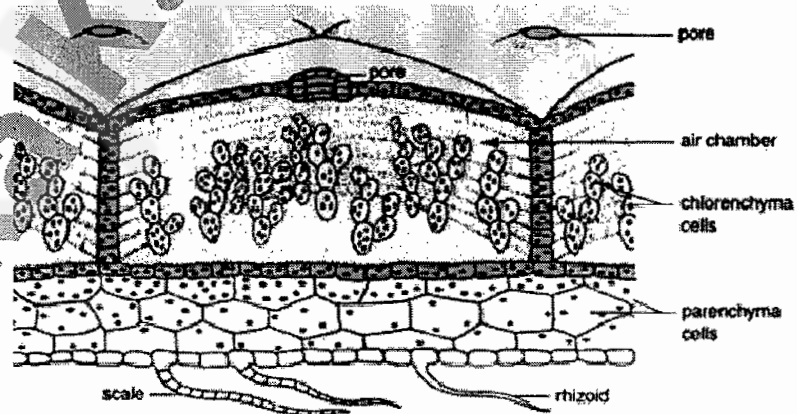


Figure 2: Internal differentiation of gametophyte

Evolutionary comment: It is believed that the thalloid form might have evolved from leafy form

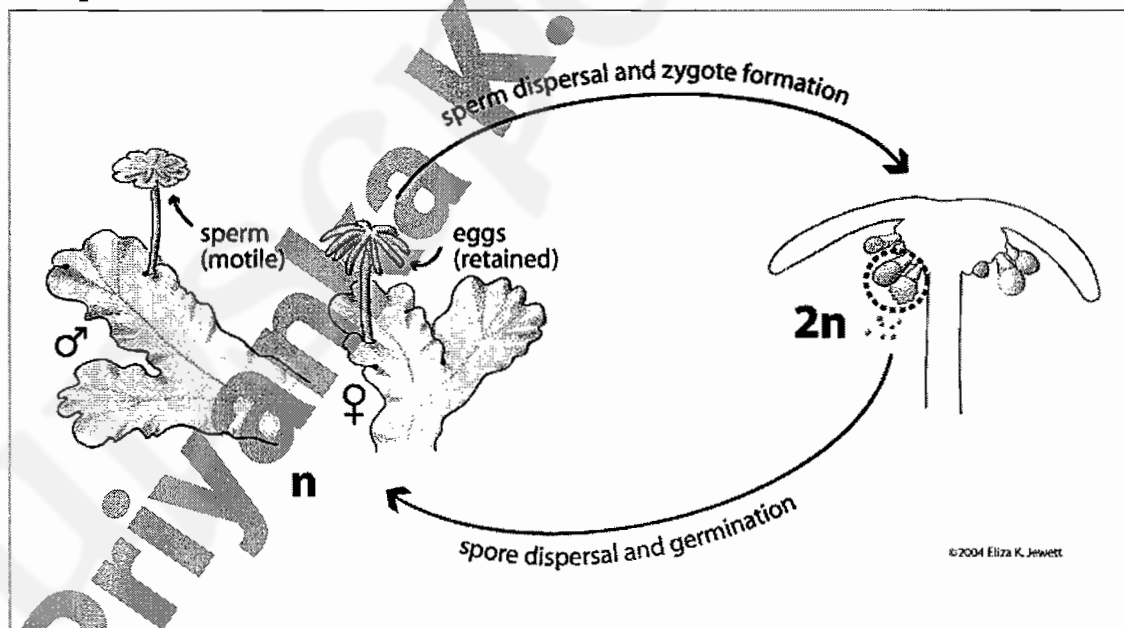
of liverworts. *Schiffneria*, a member of order Metzgeriales represents a transitional stage in between thalloid and leafy forms. In this genus, the vegetative plant body is thalloid but at reproductive stage the short abbreviated leafy shoots bearing sex organs are produced from the ventral surface of thallus. Despite a thalloid appearance, the gametothallus of *Marchantia* is considered to be very advanced among the liverworts due to elaborate internal differentiation, gametophores, areolae, two types of rhizoids with two different functions and specialised method of vegetative proliferation.

Gemmae

- They are organs of vegetation reproduction
- Produced in autumn and spring inside small **gemma cups** on the dorsal surface in the region of midrib. Each gemma cup is 0.1–0.3 cm in diameter.

- It has a membranous smooth, fringed, toothed or wavy (*M. palmate*) margin.
- From the floor of the gemma cup arise numerous erect **gemmae** of various ages inter mingled with club shaped **mucilage hairs**
- Each **gemma** consists of a multicellular green body and a unicellular hyaline **stalk** with which it is attached to the base of the gemma cup.
- The body of the gemma is elliptical and flattened like a double convex lens.
- The gemma is 4 to 5-celled thick towards the middle and gradually thins out to become single layered towards the margins.
- The margin bears two shallow lateral and opposite notches. **Growing points** are situated in these notches.
- Most of the gemma cells are **chlorenchymatous** (i.e., contain chloroplast).
- A few individual cells, just below the margin may possess oil bodies instead of chloroplasts. They are called **oil cells**.
- Some larger colourless cells containing denser granular cytoplasm are also scattered on both the flat surface of the gemma. These are known as **rhizoidal cells** as they can produce rhizoids after falling on the moist substratum.
- The gemma may also possess few **mucilage cells** for retaining moisture. At the base of the gemma there is another notch where the hyaline stalk cell is attached. The cells of gemma, bordering this notch, have very weak walls.
- The mature gemma breaks its connection from the stalk cell due to (i) rain drop splash, (ii) pressure of the young growing gemmae, (iii) swelling of the mucilage hairs and (iv) water tensions created in the gemma cup by the secretion of mucilage hairs.
- Splashing rain drops may throw the gemmae upto a distance of one meter. They are further carried away by water currents and animals.
- On reaching suitable soil, a gemma develops smooth-walled rhizoids from its lower surface. One or both the growing points become active.
- When both the growing points are active, two thalli are formed by death and decay of the middle portion.

Sexual Reproduction



Marchantia is mostly but not always dioecious i.e., male and female sex organs occur on different thalli. (Androgynous receptacles, bearing first the archegonia and later on antheridia, have been reported in a couple of species e.g. *M. grisea*)

Sexual reproduction is **oogamous**. Sex organs develop during winter to spring in India. Sexual reproduction is controlled by day length (photoperiod) and humidity. It can be artificially induced by long photoperiod, red light and reduction in nitrogen supply.

The sex organs are produced on discoid **receptacles** of upright branches called **gametophores**. The gametophore bearing antheridia (male sex organs) is known as **antheridiophore** and that bearing archegonia (female sex organs) is called **archegoniophore**. The gametophores arise terminally on the thallus lobes. Growth of the thallus lobe stops after bearing a gametophore.

The gametophore and receptacles show internal structure typical of *Marchantia* thallus. The stalk of the gametophore bears on its anterior side (morphologically ventral side) on or two furrows with tuberculate rhizoids and scales. The provide the receptacle with water through capillarity. The posterior side (morphologically dorsal side) often possesses air chambers but with fewer assimilatory filaments. The central cells of the stalk are narrow and vertically elongated.

Based on the following observations we can establish that Gametophores (receptacles) are mere prolongations of thallus.

1. The gametophores of *Marchantia* arise (terminally from the thallus lobes).
2. After bearing the gametophore, the thallus lobes stop further elongation.
3. The stalk of the gametophore can sometimes branch dichotomously like the lobes of the thallus.
4. The receptacle is repeatedly divided to become 8-lobed, with each lobed, with each lobe having its own growing point.
5. The stalk of the receptacle possesss air chambers on the posterior side and grooves containing rhizoids and scales on the anterior side. The two sides correspond to upper and lower surfaces of the thallus.
6. The internal structure of the receptacle is similar to that of the vegetative thallus in having photosynthetic region on the upper surface and storage region on the lower surface. The lower surface similar bears rhizoids and scales.

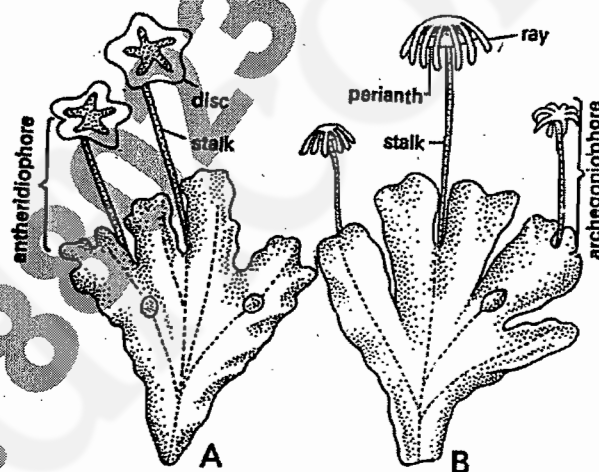


Figure 3: Reproductive branches

The male receptacle is commonly 8-lobed and slightly concave on the upper side. The upper surface bears many papilate elevation (of osstioles) in radial rows. Internally the receptacle has an upper photosynthetic region and lower storage region. Photosynthetic region consists of an upper epidermis having air pores which open into triangular air chambers having assimilatory filaments. The

antheridia occur inside **antheridial chambers** which form 4-8 radiating rows depending upon the number of receptacle lobes. Each radiating row contains 10-12 antheridia. Their arrangement is acropetal (youngest towards margin or growing point and oldest towards the centre). The antheridial chambers are flask-shaped cavities which are embedded in the upper surface of the receptacle. Each antheridial chamber has a wide cavity and narrow canal which opens dorsally by a raised pore called ostiole. In some species the wall of the antheridial chambers bears **mucilage filaments** which keep the antheridial chamber moist.

The antheridium arises singly from the floor of an antheridial chamber. It is ovoid (globose to conical) in outline. The antheridium is attached to the base of the chamber by means

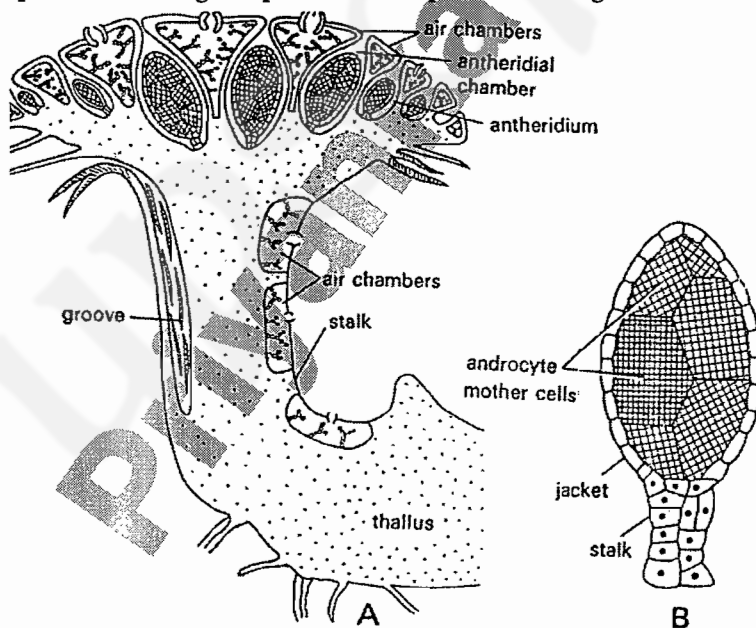


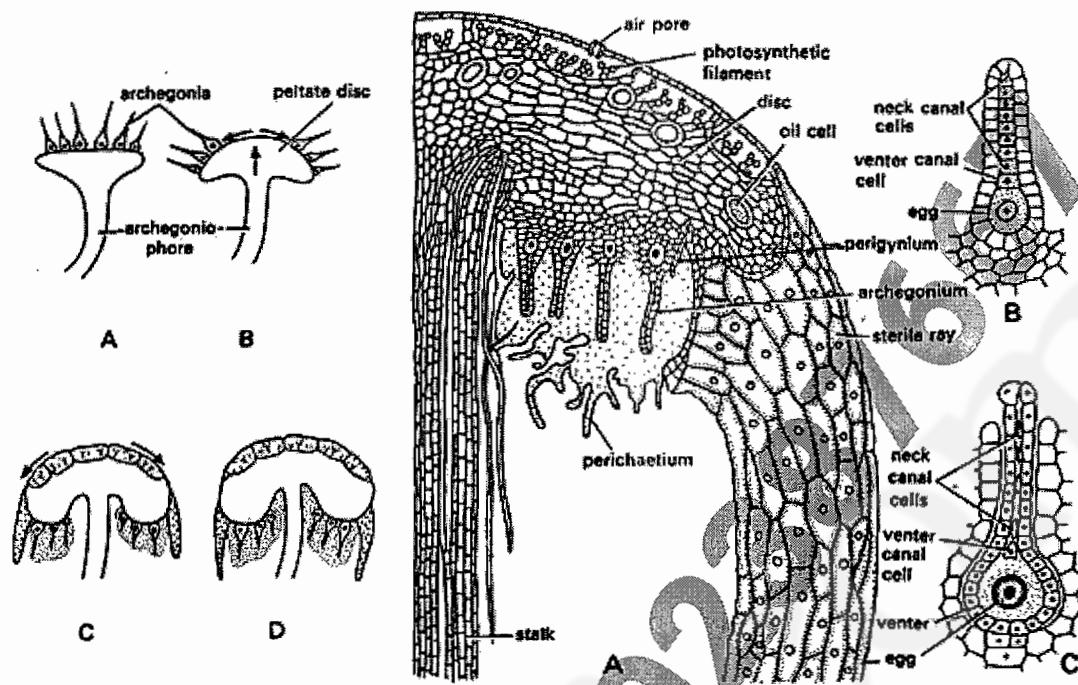
Figure 4: Antheridiophore and Antheridium

of a short but Multicellular stalk. The antheridium is covered by a single layered wall or jacket. Inner to the jacket are present compactly arranged cubical cells with large nuclei and dense cytoplasm. These cells are **androcyte mother cells**. Each androcyte mother cell divides diagonally to produce two **androcytes** or **spermatogenous cells**. The protoplast of each androcyte gives rise to a single spermatozoid.

The rain or dew water present on the slightly concave upper surface of the male receptacle is drawn into the antheridial chamber. The androcytes and the jacket cells of the antheridium swell up and exert pressure against the wall of the antheridial cavity or chamber, which causes the upper jacket cells to break down. The mass of androcytes, along with their mature spermatozooids, is liberated from the ruptured antheridium in a column of mucilage. It comes to the upper surface of the receptacle where the mature spermatozooids are released in a few minutes.

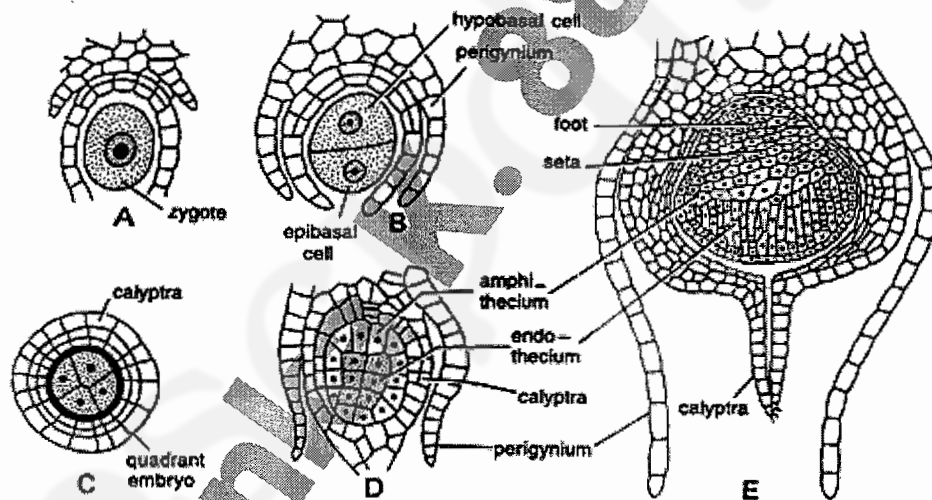
Archegoniophore. It consists of stalk and a female receptacle. The stalk is very short before fertilization but it elongates thereafter 2-8 cm. It is stouter than that of the antheridiophore. The stalk bears 1-2 grooves having scales and tuberculate rhizoids.

The female disc or receptacle is eight lobed. In many species the female receptacle contains nine finger-like cylindrical and green processes called **rays**. Seven of them lie alternating with the lobes while two occur at the margins between two lobes. In the young condition the rays are pendent (drooping downwards) so that the receptacle looks like an umbrella. In the mature condition the rays spread out horizontally to form a stellate or star shaped structure.



Marchantia : A. Position of archegonia on archegoniophore before fertilization, B-D. Stages in inversion of archegonia after fertilization.

Fig. 18 A-C. Marchantia : A. Archegoniophore in vertical section, B-C. Young and mature archegonia respectively.



Marchantia : Successive stages in the development of sporophyte.

Figure 5: Female reproductive structures

The female receptacle is generally convex on the upper surface. Female sex organs or archegonia occur in patches on the ventral surface of the lobes. Each patch contains 12-16 hanging or inverted archegonia. The younger archegonia are found bear the stalk while the older archegonia lie near the periphery of the lobe.

All the archegonia of a lobe are covered over by a two lipped hanging membrane called **perichaetium**.

Early in the development the archegonia are borne dorsally behind the growing apex of each lobe in an acropetal fashion. Later on due to overgrowth of the central part of the upper surface of the disc, the apices of the lobes along with the groups of archegonia move downwards and inwards till the growing points come to lie next to the stalk. During the inversion the younger archegonia move towards the stalk while the older archegonia appear outwards near the periphery of the lower surface

A mature archegonium is a flask-shaped structure which is attached ventrally to the lobe of the receptacle by means of a short but Multicellular stalk. The base of the archegonium is surrounded by a single layered, cup or collar like covering known as **perigynium**.

The body of the archegonium consists of a dilated basal **venter** (towards the receptacle) and long tubular **neck**. Both the neck and the venter are bounded by a single layer of sterile cells. These cells are bounded by a single layer of sterile cells. These cells are arranged irregularly in the wall of the venter but in the neck they are arranged in **six longitudinal (vertical) rows**. The distal end of neck is closed by **4 lid cells**. The neck possesses a tubular **neck canal**. The latter has an axial column of usually **4-6 neck canal cells**.

The dilated venter contains a large naked **oosphere** and **venter (ventral) canal cell** above it. The oosphere possesses a large nucleus and dense cytoplasm containing a **receptive spot** at one end.

Fertilization

At this time stalk of the archegoniophore is very small. Fertilization requires moisture and presence of both male and female plants nearby. The rain drops falling on the slightly concave surface of the male receptacle. Splash the spermatozooids upto 60 cm. If the archegonia are upright, the splashed antherozoids may directly move to the necks of the archegonia where a drop of mucilage is present. Or, if the archegonia are hanging downwards, the convexity of the upper surface of the female receptacle may help in sliding the antherozoids to the under surface. Furrows on the stalks of the archegoniophore aid in the upward movement of the spermatozooids by capillarity. Heavy dew keeps the surfaces of thalli moist for transportation of spermatozooids. The spermatozooids reach the archegonia by directly swimming to them if the female thalli are submerged in water. Mites visit both male and female receptacles for sucrose secreted by their sex organs. They transport spermatozooids from the antheridia to the archegonia.

After reaching the female disc the antherozoids are attracted to the interior of archegonia by certain proteins or potassium salts released by the oospheres (chemotaxis). One antherozoid fuses with the oosphere to form a diploid zygote which develops a wall and is called oospore. Many oospores are produced in a female receptacle but only a few form sporogonia due to the limitation of food and space.

Sporogonium

The sporogonium is a diploid hanging structure which is covered by three gametophytic coverings viz., calyptra, perianth and perichaetium. Calyptra is 1-4 layered and is formed by the enlargement of venter wall. Perianth or pseudoperianth is one-layered and is developed from perigynium. Perichaetium is a fimbriated bilipped covering over the receptacle lobe.

The mature sporogonium is differentiated into three parts—**foot, seta and capsule**. The foot is basal part of the sporogonium which takes part in its fixation and absorption of nourishment from the female receptacle. It is embedded in the female receptacle. The foot is a swollen multicellular structure which is bulbous or anchor-like. Its cells are thin-walled and angular. The foot cells, which are in contact with the tissue of the female receptacle, are lobed to function as haustoria.

The **seta** connects the foot with the capsule. It consists of thin-walled and vacuolated parenchymatous cells which are arranged in the form of vertical rows. Seta conducts food from foot to the capsule. Initially the seta is short but at maturity it elongates suddenly due to enlargement of its cells. The elongated seta pushes the capsule out of the three investing sheaths. At this time it may measure 12 cm in length.

The **Capsule** is oval to globular in outline. It is broader than the seta. The mature capsule is yellowish or brownish in colour. Its wall or jacket is single layered except at the apex where, in certain species, some of the internal cells may remain sterile (e.g., *M. chenopoda*).

The jacket encloses a mass of fertile **sporogenous cells** and sterile **elater mother cells**. The elater mother cells elongate and lose their protoplasm to form empty spindle-shaped brown hygroscopic cells called **elaters**. They have two or three spiral bands of thickenings on their inner walls. Along with the changes in humidity, the elaters show twisting movements due to the expansion or contraction of bands of spiral thickenings in different direction. In the young condition they may conduct water nutrients from the seta to the spore mother cells.

Each sporogenous cell produces 8-32 spore mother cells. Each of them divides meiotically to produce 4 haploid spores. The process is called **sporogenesis**. Out of the four spores of a tetrad, two are genetically male while the other two are female. The elaters and the spores are distributed evenly in the capsule.

Nutrition of Sporogonium. The sporogonium of *Marchantia* was previously considered to be a **total parasite** because of the absence of chloroplasts in the mature state, ventral position and the covering sheaths which can prevent light penetration. However, the young sporogonial cells have been found to contain abundant chloroplasts with enclosed starch grains (Thomas *et al*, 1979) showing that the cells perform active photosynthesis. Young sporogonium seems to depend upon the gametophyte for water, minerals and some organic substances which it cannot manufacture. The young sporophyte of *Marchantia* is, therefore a **semi-parasite** while the old one may be a **total parasite**.

8. Genus: Anthoceros

Basic introduction

The class of Anthocerotopsida consists of only about 100 species in six genera, the most familiar being *Anthoceros* sp., generally occurs in moist, shaded habitats in sub-tropical and warm temperate regions. The gametophyte of *Anthoceros* sp. superficially resembles that of the thallose liverworts. They have a strong dorsoventral orientation; are often rosette-like and are usually less than 2 cm. in diameter. In addition, *Anthoceros* sp. have extensive internal cavities filled with mucilage, rather than air, as in the gametophytes of the thallose liverworts. These mucilage-filled cavities are often inhabited by cyanobacteria of the genus *Nostoc*, which supply nitrogen through nitrogen fixation to their host plants.

Some species of *Anthoceros* are unisexual and others are bisexual. The antheridia and archegonia are sunken on the dorsal surface of the gametophyte. Numerous sporophytes may develop on the same gametophyte.

The sporophyte of *Anthoceros* sp. consists of a foot, and a long cylindrical sporangium. Very early in its development, a meristem, or zone of actively dividing cells, develops between the foot and the sporangium; this meristem is active as long as conditions are favourable for growth. As a result, the sporophyte continues to elongate for a prolonged period of time. Dehiscence of the sporangium begins at its tip and spreads toward its base as the spores mature. The dehiscing sporangium splits longitudinally into ribbon-like halves. The sporophyte contains several layers of photosynthetic cells. Its surface is covered with a cuticle and contains stomata. A small tubular growth of gametophyte tissue, called an "involucre" grows upwards around the base of the sporophyte.

Genus Description

Anthoceros is worldwide in its distribution. It commonly occurs on soil both in the tropical and temperate regions. It has over 200 species with 25 species being reported from India. The three common Indian species which occur in the Himalayas are: *Anthoceros himalayensis*, *A. erectus* and *A. chambensis*.

Gametophyte

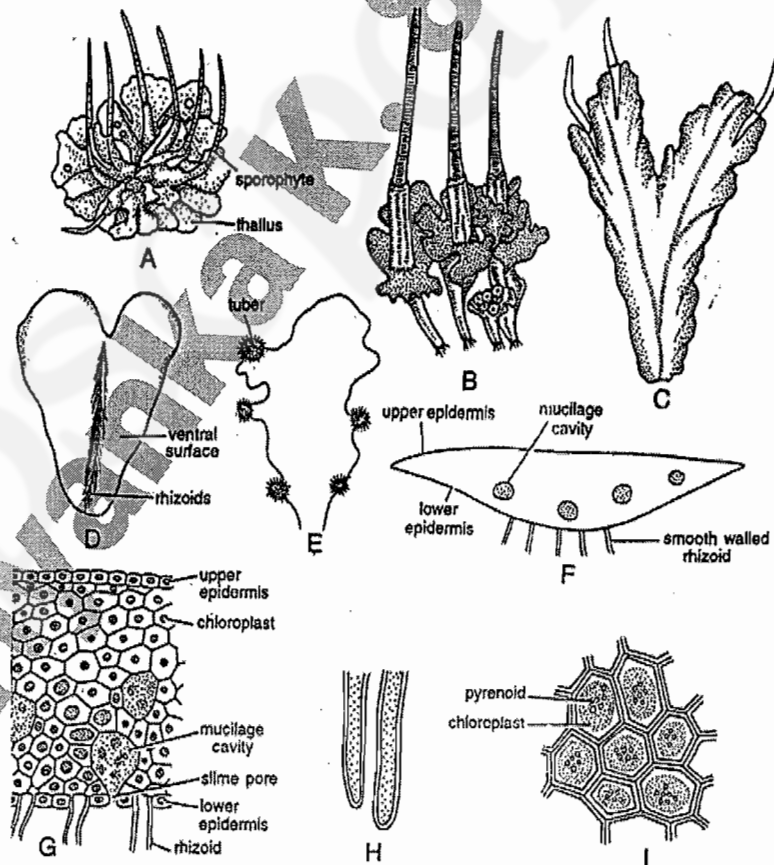


Figure 1: Gametophyte features of *Anthoceros*

The adult gametophyte plant is a small, yellowish green or dark green dorsiventrally flattened, lobed thallus, resembling that of a thalloid liverwort. The lobes are thick and fleshy with folded margin. There is no distinct midrib. The upper surface of the thallus is smooth in some species (*A. laevis*) but rough in others (*A. fusiformis*) due to the presence of ridges.

The ventral surface lacks scales, tuberculate rhizoids and mucilage hairs. However, it bears numerous, unicellular, smooth-walled rhizoids. These anchor the prostrate thallus to the substratum. In addition small, rounded bluish green thickened areas can be spotted on the ventral surface of the thallus. These thickened spots are Nostoc colonies.

Anthoceros erectus has generally a different habit. The thick, fleshy thallus of this species is often raised on a thick, upright or ascending, stalk-like structure. The latter expands above into a cap-like structure.

In the months of September and October the *Anthoceros* thallus bears long, cylindrical sporogonia. They arise in clusters from the dorsal surface of the thallus. Each sporogonium has a tubular sheath around it at its base. It is the involucre.

Internally the thallus is several layers of cells thick but without a midrib. There is no tissue differentiation and little cell specialization. Thus the assimilatory and storage regions are not recognizable. The entire thallus consists of soft parenchyma. The cells are uniform and compact. Air containing channels or air chambers and air pores are lacking. The surface cells of the thallus are smaller, each with a comparatively large lens-shaped chloroplast. They are, however, not cuticularized. There is thus no organized epidermis.

In some species of *Anthoceros* there are stoma-like pores or slits on the ventral surface of the thallus. These are called the **slime pores**. Each slime-pore is guarded by two bean-shaped guard cells with thin walls. The guard cells do not function to control the size of the pore which thus remains completely open. The slime pores lead inwards often into large schizogenously formed, rounded intercellular spaces or cavities, which are filled with slime (mucilage), not air. Very often these mucilage cavities are inhabited by *Nostoc* – a blue green alga. Hormogones of *Nostoc* gain entry into the mucilage cavities through the slime pores. There they multiply and form colonies. The cavities containing *Nostoc* colonies are visible to the naked eye as small, deep blue-green rounded spots or specks on the under side of the thallus.

Sexual Reproduction

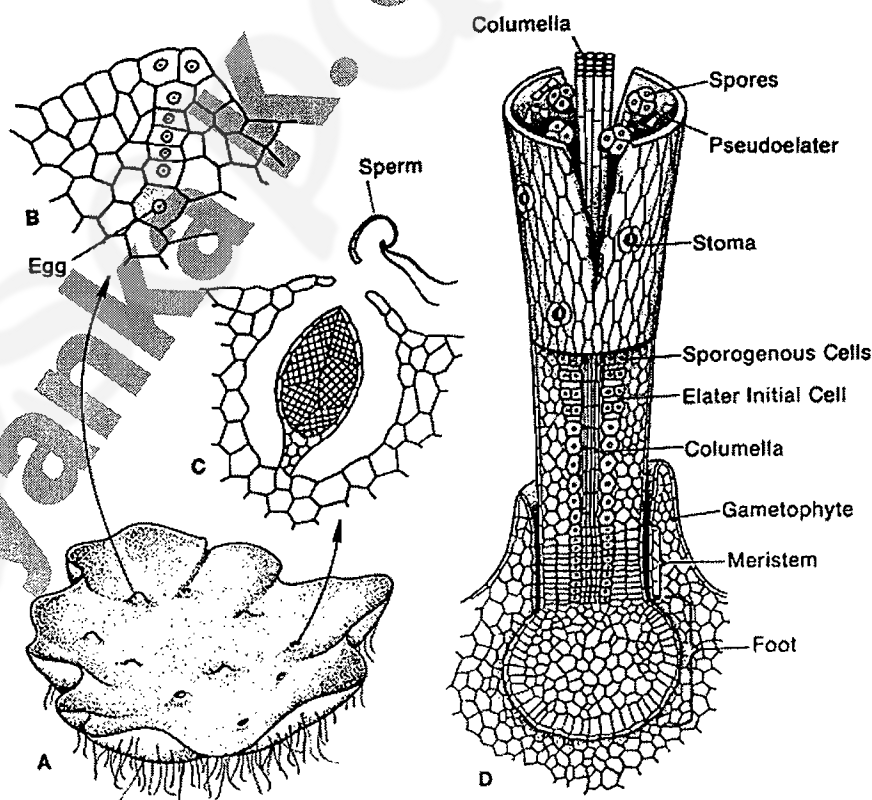


Figure 2: Reproductive structures. A. Gametophyte, B. Archegonium, C. Antheridium, D. Sporophyte

Anthoceros thallus is concerned with vegetative reproduction and the sexual process. Many species of *Anthoceros* are **monoecious**, other are **dioecious**. *A. gollani*, *A. longii*, *A. fusiformis* and *A. punctatus*. *A. crispus* and *A. himalayensis* are monoecious but protandrous. The antheridia appear much earlier than the archegonia. The common dioecious species are *A. erectus*, *A. chambensis*, *A. hallii*, *A. pearsoni* and *A. laevis*.

The sex organs in *Anthoceros* are immersed in the thallus tissue on the upper surface. The sexual development occurs under short day conditions.

Antheridia: The antheridia of *Anthoceros* are unique in being normally **endogenous**. They occur singly or in groups on the upper surface of the thallus within closed cavities called the **antheridal chambers**. Each antheridial cavity is roofed over by the thallus gametophyte tissue two cell layers in thickness. The ripe antheridia are bright orange in colour. Each antheridium has an ovoid or pouch-like body. It is raised on a multicellular, short or long, slender stalk. The latter usually consists of four vertical rows of cells. In *A. laevis* it is thicker. The body of the antheridium, as in the liverworts, consists of a **jacket layer** or an **antheridial wall** enclosing a mass of **androcytes**.

When the antheridia reach maturity, the roof of the antheridial chamber bursts open irregularly. The antheridia thus exposed dehisce following absorption of water presumably by the mucopolysaccharides in the matrix surrounding the sperm mass. This leads to rupture at the distal end of the swollen antheridium. An aperture is thus formed at the distal end of the antheridium, through it oozes out the matrix containing the sperm vesicles. Finally the sperms are discharged in the water that caused the rupture by dissolution of the walls of the sperm vesicles. The liberated sperm is a tiny, biflagellate structure. The two flagella are equal and almost of the same length as the body. They are inserted at the slightly broader, anterior end of the slender body which is slightly curved.

Archegonia: The archegonia of *Anthoceros* are sunk deep in the fleshy thallus on its upper surface. They lie close to the growing point. They are developed in **acropetal** order. In the monoecious species the archegonia appear later, on the same thallus which produced antheridia.

Each archegonium consists only of an **axial row** of usually four to six **neck canal cells**, a **ventral canal cell** and an **egg**. There is no sterile **jacket layer** except the distal rosette of **cover cells** forming its tip. The cover cells slightly project above the general, upper surface of the thallus where it is usually surrounded by a somewhat funnel-shaped mass of mucilage called the **mucilage mound**. The surrounding vegetative cells of the thallus offer protection to the cells of the axial row. The archegonium of *Anthoceros* immersed in the thallus and in direct contact with the surrounding vegetative cells differs from that of all other Bryophytes and resembles certain of the Pteridophytes.

Sporophyte

The zygote is the first structure of this phase. By repeated segmentation it develops into an elongated **embryo**. The embryo by further cell division, cell differentiation and continued growth rapidly grows into an elongated, spindle-shaped **sporogonium** [or the **sporophyte**] with a bulbous base. The sporophytes usually grow in clusters from the upper surface of the thallus, each surrounded at its base by a tubular **involucre**. The unstalked, horn-like sporogonium ranges from one to several centimeters in length. It is an outgrowth from the thallus and thus is a gametophytic structure.

It is differentiated into three regions; (i) the **capsule**, (ii) the **intercalary or intermediate zone** and (iii) The **foot**. The seta is absent. Its place is taken up by the intercalary zone which is **meristematic**. It is protective in function and also gives support to the weak intercalary zone.

I. Capsule. It forms the major and conspicuous part of the sporophyte. In form it is long, slender; smooth, upright and cylindrical. It is nearly of uniform thickness throughout its length except towards the apex where it slightly tapers. Usually it is 2 to 3 cms. or in some species up to 15 cms. long. It is light green at first but turns grey or brown towards maturity. Potentially the sporogonium (capsule) of *anthoceros* is capable of unlimited growth in length because of the presence of meristematic zone at the junction of the foot and the capsule.

Internally the capsule shows great elaboration and complexity of structure. In the center of the capsule is a slender solid core of sterile tissue. It is the **columella**. The cells constituting it are narrow, elongated - arranged in sixteen vertical rows. It is **endothelial** in origin.

Around the columella is a double layer of elongate but domed **sporogenous tissue**. It is in the form of a cylinder between the columella and the capsule wall. It extends over the top of the columella like a dome - a feature in sharp contrast to the liverworts. In the respect *Anthoceros* sporophyte resembles *Sphagnum* moss. The sporogenous tissue originates in the meristematic zone where it is single layered and is called the **archesporium**. Higher up it becomes a two-layered sporogenous tissue.

Thus at successive higher levels spore mother cells, spore tetrads and meiospores are formed. Among the spore tetrads and mature spores are found the **pseudoelaters**. They form chains of one to four elongated thin or thick-walled, sterile cells of irregular shape. The pseudoelaters are smooth-walled and are

nutritive in function. They lack spiral thickenings and are pluricellular, sometimes unicellular. When they dry up, they act hygroscopically also.

External to the fertile zone is the **capsule wall**. It is several layers (usually 4 to 6) of cells in thickness. The outermost layer of wall is the **epidermis**. It consists of narrow, vertically elongated cells with their outer walls cutinized. Here and there the epidermal layer is punctured by **stomata** similar to those of the higher plants. Each stoma consists of a **pore** surrounded by two **guard cells**. The cells of the capsule wall within the epidermis are **chlorenchymatous**.

II. Intermediate or intercalary zone. It is a narrow zone of **meristematic cells** located at the base of the capsule just above the foot. The meristem constantly adds new cells to the capsule at its base. They become progressively differentiated into columella, archesporium and capsule wall. The presence of a basal intercalary meristem enables the capsule to grow for a long period and form spores.

III. Foot. It is a rounded bulbous structure deeply embedded in the tissue of the thallus. By means of the foot *Anthoceros* capsule is well anchored upon and attached to the thallus. The foot mainly consists of a mass of parenchymatous cells. The surface cells of the foot, however, often surface of the foot and penetrate the tissue of the thallus. The foot of *Anthoceros* sporogonium is thus specialized to function as a **haustorium**. It absorbs food and water from the parent thallus for the sporophyte. The region of contact between the foot and the thallus tissue is well marked in many species. It is called the **placenta**. The cells on the gametophytic side of the placenta are "transfer cells". These cells develop long thread-like ingrowths which branch and the branches anastomose to form wall labyrinths. The plasma membrane in these cells follows the contours of the wall and the cell cytoplasm penetrates between the individual ingrowths or into the interstices of the labyrinth. The plasma membrane in the transfer cells is thus greatly increased.

The capsule dehisces basipetally usually by two valves which curl back exposing the mass of spores intermixed with pseudoelaters on the central column.

9. Genus: Sphagnum

General characteristics of the Sub-Class Sphagnidae

The subclass-Sphagnidae includes the mosses which are often very important constituents of peat bog vegetation. Hence the name *peat* or the *bog mosses*. They grow in extensive masses on boggy and peaty soils and also as submerged aquatics in peaty pools. The subclass sphagnidae is characterized by the following distinctive features :-

- The simple, flat, plate-like thallose **protonema** is fixed to the substratum by numerous **rhizoids**.
- The rhizoids are multicellular. The septa between the cells are oblique.
- The upright, **leafy branch** originates from a single protomenal cell. It grows by means of a three-sided **apical cell** into the adult or mature plant. Also called the gametophores.
- Usually a single gametophore develops from one protonema.
- The leaf of adult bog moss has a unique structure. It consists of two kinds of cells, the narrow, living green **assimilatory cells** and the large, colourless, dead **capillary cells**.
- The leaf has **no midrib**.
- The antheridia occur singly and are **axillary** in position.
- They develop on special **side branches**. The antheridial branches are relatively strong than the vegetative shoots.
- The archegonia are **terminal** in position. They occur in clusters of three usually at the apices of short **female branches** from among a crown at the top of the plant.
- The mature sporogonium is differentiated into an enlarged **foot**, a rudimentary **constriction like seta** and a large, rounded **capsule** which is globular in form.
- The young capsule is invested by the calyptra.
- The leafless, stalk-like **pseudopodium** carries the ripe sporogonium at its top.
- The columella develops from the entire **endothecium**. It occupies the major part of the cavity of the capsule.
- The **sporogenous tissue** develops from the inner layer of the **amphithecium**.
- The central, hemispherical columella is capped by the domeshaped spore sac.
- The ripe capsule dehisces by the separation of a disc-shaped **lid or operculum** at its top.
- The peristome is absent.
- The basic chromosome number in *Sphagnum* is $n = 19$, with of course, a varying number of tiny bodies, the so-called *m* chromosomes.
- The sub-class Sphagnidae includes a single order Sphagnales with a single family Sphagnaceae represented by single genus *Sphagnum*.

Genus: Sphagnum

Habitat & Ecology

Sphagnum is an interesting genus of the mosses. No other group of mosses is as ecologically dominant or economically important as the *Sphagnum* species are as a group. The genus includes more than 336 species. Of these 32 have been reported from India. They grow in wet or very wet places as semiaquatics and also as submerged aquatics confined to acidic water logged sites that are poorly mineralized as well. As a group these water loving *Sphagnum* species (hydrophytes) possess some adaptations for dealing with periodic drought conditions. For this reason some workers term them **xerophytic hydrophytes**. They thrive best in cold bogs and marshes of higher latitudes. The individual *Sphagnum* plants grow closely matted together forming extensive masses. The latter form a continuous and complete vegetative spongy cover over the surface of water in acid pools, ponds and lakes converting them to quaking bogs of peat lands. It is dangerous to traverse the quaking bog. *Sphagnum* is intolerant of lime. The bog water is antiseptic. Owing to its germicidal properties and deficiency of oxygen it acts as a preservative. Plants, animals, logs of wood and even human bodies entrapped beneath surface in the quaking bogs have been recovered well preserved after centuries.

Sphagnum takes up mineral nutrients (e.g. Ca, Mg, Na, etc.) from water and uses them to lay down new tissue and grow. The tissue may hold these minerals even when it dies, so *Sphagnum* peat systems are often sinks for minerals as long as the plant tissue remains wet and does not decompose.

The growth of *Sphagnum* increases the acidity of its fluid environment. Skene (1915) attributed it to the process of selective ionic absorption. Rose (1953) reported that the pH in the interior of tufts is usually lower as compared with the surrounding water. *Sphagnum* can acidify an area by exchanging hydrogen ions for base cations (K^+ , Na^+ , Ca^{++} , Mg^{++}). *Sphagnum* plants use cation exchange to obtain macro and micro nutrients that are often in low concentrations in permanently inundated habitats. The low mineral nutrient and acidic environment that results is not tolerated by many vascular plants, so there is very little competition for resources. Cation exchange is accomplished in *Sphagnum* cell walls by uronic acid (Clymo and Hayward 1982).

An acidic, cold, constantly saturated, and mineral-poor environment impedes decomposition of the older portions of the *Sphagnum* plant below the growing apical tip. Growth and reproduction of bacteria and fungi that normally mediate decomposition are limited or prevented under these acidic, saturated, cold, and low-oxygen conditions. An additional phenolic microbial inhibitor, called **sphagnol**, is produced by most *Sphagnum* species (Clymo and Hayward 1982). Farmer and Morrison (1964) have also confirmed the presence of unusual lignin containing p - hydroxyphenyl units in various species. The lack of decomposition results in the accumulation of peat. The rate of accumulation varies considerably with water chemistry, pH, *Sphagnum* species, and moisture regime (wet vs. dry). As the plant grows its basal older parts die. The dead portions, accumulate from year to year as partially decompose material which gradually fills the pond or the lake. These deposits may as well contain the remains of other plants growing there such as the sledges, heathers, cotton grass etc. The oldest deposits in western Washington date to the last glaciation and are therefore about 10,000 years old. *Sphagnum* has been used over the centuries for its antiseptic and absorptive properties. It has been used for dressing wounds, as packing material, for lamp wicks, and as an amendment for increasing the water-holding capacity and acidity of soil.

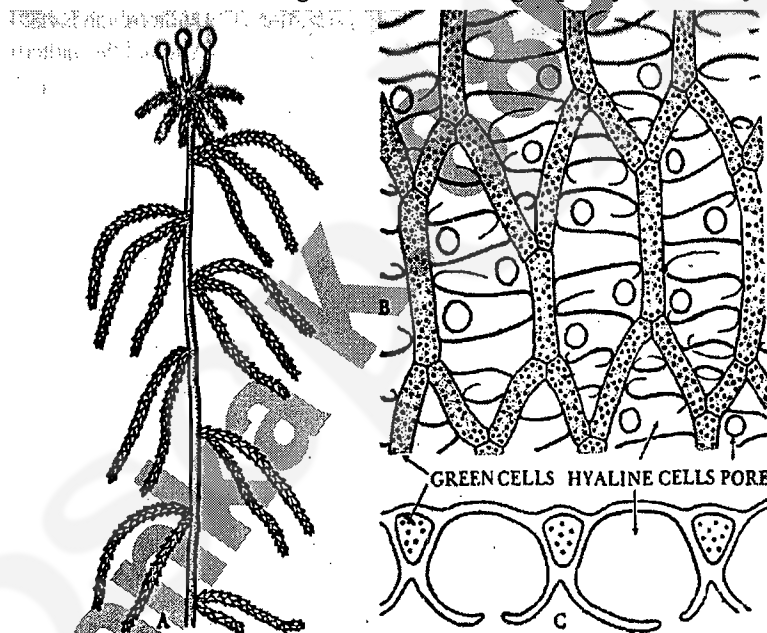


Figure 1: Morphology of *Sphagnum*. A. gametophyte with various branches; B. surface view of a mature leaf; C. sectional view of a mature leaf

The genus *Sphagnum* has been divided into ten Sections based on cortical cell anatomy, hyaline cell anatomy, number of branches per fascicle, branch color, branch leaf shape, position of green cells, the presence of fibrils, and habitat preference.

Sphagnum sp. are believed to have played a very important role of pioneers during the period just after the last glaciation.

Mature gametophytic structure

The genus *Sphagnum* has been divided into ten Sections based on cortical cell anatomy, hyaline cell anatomy, number of branches per fascicle, branch color, branch leaf shape, position of green cells, the presence of fibrils, and habitat preference.

Sphagnum plant generally is an erect perennial. The axis is branched in a monopodial manner. It lacks rhizoids at maturity. The conspicuous, leafy *Sphagnum* plant is the gametophyte generation which is haploid. It produces the egg and sperm either on different branches of the same plant (monoecious) or on different plants (dioecious).

Sphagnum mosses differ from other mosses in many ways. One important diagnostic feature is the organization of the branches on the plant. There are 4 types of branches: 1. Pendant Branches 2. Divergent Branches 3. Comal Branches 4. Innovations [Meant for vegetative propagation].

Branches are arranged in clumps called fascicles that consist of two or more spreading [divergent] branches and one or more pendent branches. The number of pendent and spreading branches are used in species identification. *Sphagnum* grows apically (from the top). The young branches are usually packed into the top of the plant in a feature called a capitulum or coma. The branches present on the coma are called Comal Branches. The shape of the coma can be used for species identification (Sastad and Flatberg 1994).

Most *Sphagnum* mosses are anisophyllous, meaning they have two kinds of leaves-- those found on the branches and those found on the main stem. The two kinds of leaves are most easily differentiated under ideal growing conditions. Less-than-ideal conditions (such as insufficient moisture) can result in less differentiation between the branch and stem leaves (hemi-isophyllous). Plants that grow under conditions of fluctuating water levels can have branch and stem leaves that are undifferentiated (isophyllous). Since stem and branch leaves are most often used for species identification, the stem leaves of two different species that are in an isophyllous form can be nearly identical, so species identification can be difficult to impossible in specimens from less-than-ideal moisture and mineral regimes.

Anatomy: The main stem of the *Sphagnum* plant is composed of a central region surrounded by one to five layers of hyaline cells, or may lack a differentiated cortex. In the hyalodermal region / cortical region some open retort cells are found, which play a role in water regulation.

The leaves are arranged with the large, dead, hollow hyaline cells interspersed with smaller live photosynthetic or green cells. The morphology of *Sphagnum* hyaline cells enables them to retain large amounts of water. They die at maturity, and in many species are thickened with annular-helical ridges (spiral fibrils) and are frequently perforated by pores with edges that may be thickened. These hollow spherical cells hold water like a vase. Some species of *Sphagnum* have been shown to hold 16 to 26 times their dry weight in water. Water will wick up both the stem and pendent branches, filling each hyaline cell and then wicking up the cell wall of the next hyaline cell and spilling into and filling it (McQueen 1990). In this way, *Sphagnum* wicks and stores water and remains moist even feet above the water table. Species that grow in wetter habitats have weaker stems and are generally limp, with fascicles spaced widely along the stem. Species of drier habitats have more rigid stems, with fascicles closer together along the stem. Species growing above the water table are often brighter pigmented (not all green). The branch arrangement and density is dependent on the water table throughout the year, and summer conditions may not be an indicator of yearly conditions.

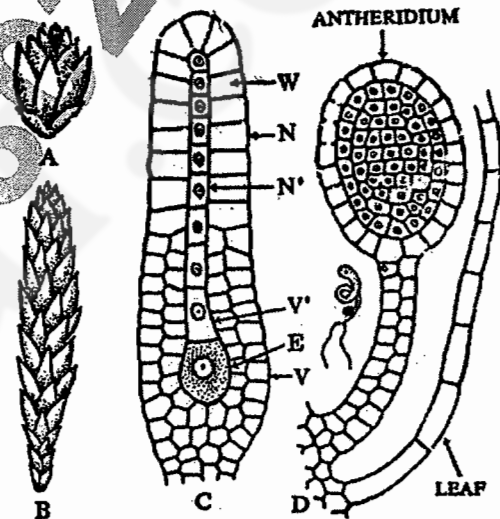


Figure 2: *Sphagnum*: A. Archegonial branch B. Antheridial Branch C. Archegonium D. Antheridium

Sexual reproduction

Sphagnum gametophyte produces by two methods usual for all the bryophytes: these are vegetative and sexual reproduction.

Mature *Sphagnum* plants produce sex organs in favorable situations. They are formed in autumn on special, short, densely leafy and slightly modified branches. The sexual branches either occur in the terminal branch cluster or laterally. Even in the monoecious species the two kinds of sex organs never occur on the same branch. The antheridial branches appear first. The sex organs are formed in abundance. Paraphyses are always absent.

Antheridial branches are usually shorter but stouter than the vegetative branches. They are spindle-shaped and resemble small catkins. They are strongly pigmented and often densely clothed with red, purple, brown or yellow leaves generally small than the foliage leaves. The position of the antheridium on an antheridial branch is shown in the following diagram.

The **archegonia** are terminal on the specialized archegonial branches. They usually occur in small groups. Typically there are three archegonia in the group. The number, however, varies from 2 to 5. The central or the middle archegonium in the cluster grows directly from the **apical cell** of the branch. It is called the **primary archegonium** and is the first to be formed. The others in the cluster are called the **secondary archegonia**. They are developed from the last segments cut off by the apical cell. The secondary archegonia are thus formed about the base of the primary archegonium. The mature archegonium is relatively a large structure. It is stalked. The stalk is fairly long. The body of the archegonium consists of a long twisted **neck** and a massive **venter**. The neck consists of six vertical rows of **neck cells**. The neck canal contains numerous **neck canal cells**. The venter and the lower portion of the neck is 2 to 3 layers of cells in thickness. The venter cavity contains a small ovoid egg.

Sporophyte phase

This phase in the life cycle ushers with the act of fertilization. The diploid zygote is thus the pioneer structure of this generation. The zygote encased in the venter, increases in size, secretes a wall around it and starts dividing. By repeated division it develops into an embryo which by further division, differentiation and growth forms the young sporogonium or sporophyte.

The ripe sporogonium or sporophyte consists of a **foot** and a **capsule**. The two are linked by a small, narrow, neck-like constriction which represents the suppressed **seta**.

Foot. It is an enlarged, bulbous structure which absorbs nutrition for the developing sporophyte from the gametophyte. It is embedded in the tissue of the dilated apex of the **pseudopodium** which develops as a prolongations of the tip of the archegonial branch after fertilization when the spores are ripe for dispersal. It is short, leafless about 12 mm or a little more in length. The pseudopodium elevates the capsule far above the perichaetial leaves and the terminal branch cluster. It thus compensates for the suppression of seta and assists in spore dispersal. The pseudopodium is liable to be mistaken as seta. In fact it is a prolongation of the axis of the archegonial branch and thus a part of the parent gametophyte.

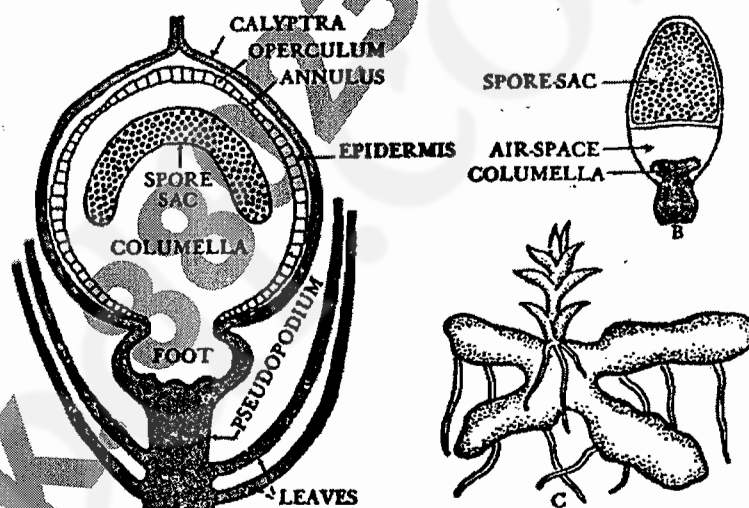


Figure 3: *Sphagnum* sporophyte

Capsule. The capsule is a small nearly globose structure. It is dark brown or black in colour when mature. It mainly consists of a massive hemispherical central **columella**. Overarching the columella is a relatively thin dome-shaped **spore sac** which contains the spores. There are no **elaters**. Surrounding the columella and the **spore sac** is the capsule wall which is 4-6 layers of cells thick. The superficial or outermost layer of the cell wall is called the **epidermis**. It consists of cuticularized cells arranged compactly. Interspersed here and there between the epidermal cells are the stomata which are rudimentary and thus nonfunctional. Each stoma consists of two guard cells. There is no stomatal aperture. The capsule wall cells contain chloroplasts but have no intercellular spaces between them. At the top of the capsule is the convex disc-shaped **operculum** or lid. The operculum is sharply marked off from the rest of the capsule region by a ring-like (circular) groove of thin-walled cells, the **annulus**.

Mechanism of dehiscence and dispersal of spores. The dehiscence of the capsule is by an explosive mechanism. It takes place on sunny days. With the formation of spore tetrads in the spore sac, cells of the columella break down. This results in the formation of a large **air cavity** below the spore sac. Under the influence of the sun the wall of the exposed, mature dark brown capsule dries and shrinks. The spherical capsule gradually becomes cylindrical. The imprisoned air in the lower half of the capsule is compressed and thus held under considerable pressure. It cannot escape. As the capsule wall shrinks the thickened lid cells resist shrinkage. A difference in tension is thus set up. This puts a strain on the thin-walled annulus cells which finally rupture under the mounting pressure of air within. Eventually the loosened small, convex lid is blown off with explosive force. The imprisoned air is suddenly released and with it the spores in a cloud. Ingold (1939) described these methods of spore discharge in *Sphagnum* as 'air gun' mechanism.

10. Sporophytes in the Genera: Funaria & Polytrichum

Sporophyte in Funaria

Morphology and Anatomy

The mature sporophyte of *Funaria* is differentiated into **foot**, **seta** and **capsule**.

The foot: The foot is a small dagger-shaped conical structure embedded in the archegonial branch.

The seta: The seta is a long, slender, more or less twisted and stalk-like structure which supports the capsule at its distal end. Internally, the seta is differentiated into a central conducting strand of thin walled elongated cells, surrounded by a cortex of relatively thick walled cells. The conducting strand extends into the columella of the capsule. It helps in the transport of nutrients and water to the capsule.

The capsule: The capsule is slightly oblique or oval at maturity. The internal structure of the capsule can be studied in longitudinal section. It has three distinct regions: (i) *apophysis*, (ii) *theca proper*, and (iii) *apical region*.

1. **Apophysis.** It is the basal sterile part of the capsule which connects the capsule with the seta. It is also called the neck of the capsule. It consists of a central conducting strand of elongated parenchymatous cells which extend down into the seta. The central strand is surrounded by loosely arranged chlorophyllous cells. The outer most layer or the epidermis is interrupted by stomata through which CO₂ acquisition takes place for photosynthesis. The presence of chlorophyllous cells in the apophysis shows the photosynthetic capability of the sporophyte. Hence the sporophyte of *Funaria* is only partially dependent upon the gametophyte.
2. **Theca proper.** It is the middle part of the capsule which lies between the apophysis and the apical (opercular) region. It has a cylindrical structure and it constitutes the fertile part of the capsule. It has the following regions.
 - a. **Columella.** It is the central pith-like cylindrical portion of the theca, composed of parenchymatous cells. The columella provides water and nutrients, required for the development of spores in the spore sac.
 - b. **Spore sacs.** The columella is surrounded externally by two elongated spore sacs. The outer wall of each spore sac is 3-4-layered, whereas the inner wall is only single layered. In the young sporophyte the spore sacs are packed with many spore mother cells. Each spore mother cell forms four haploid spores. Elaters are absent.
 - c. **Air space.** A large air cavity is present outside the spore sac. It is traversed by delicate filaments of elongated green parenchymatous cells, known as trabeculae.
 - d. **Capsule wall.** The capsule wall is composed of many layers of thin-walled parenchymatous cells. The outermost layer forms the epidermis. It is followed by a 2-3-layered hypodermis of well organised colourless parenchymatous cells. Next, there are 2-3 layers of chlorophyllous cells with intercellular spaces, which constitute the photosynthetic tissue of the capsule. The innermost layer of the capsule wall is connected with the outer wall of the spore sac through trabeculae.
3. **The apical region.** The apical region of the capsule consists of two important parts, the operculum and peristome. A constriction is present at the junction of the apical region and the theca proper. Immediately below this constriction there is a rim which is composed of 2-3 layers of radially elongated cells. The rim demarcates the upper limit of the theca proper.

The operculum is a conical lid that closes the mouth of the capsule. It is composed of 2-3 layers of thin walled cells.

The peristome lies immediately below the operculum. It is attached beneath the edge of the rim and consists of two rings of long curved triangular teeth. The teeth are simply the strips of cuticle and they are not cellular. In each ring there are 16 teeth, the teeth of the outer ring are thicker and larger, whereas those of the inner ring are smaller, colourless and delicate. The outer peristomial teeth are also characterised by the presence of transverse thickening bands. Because of this, they are *hygroscopically active*.

Dehiscence of capsule and dispersal of spores

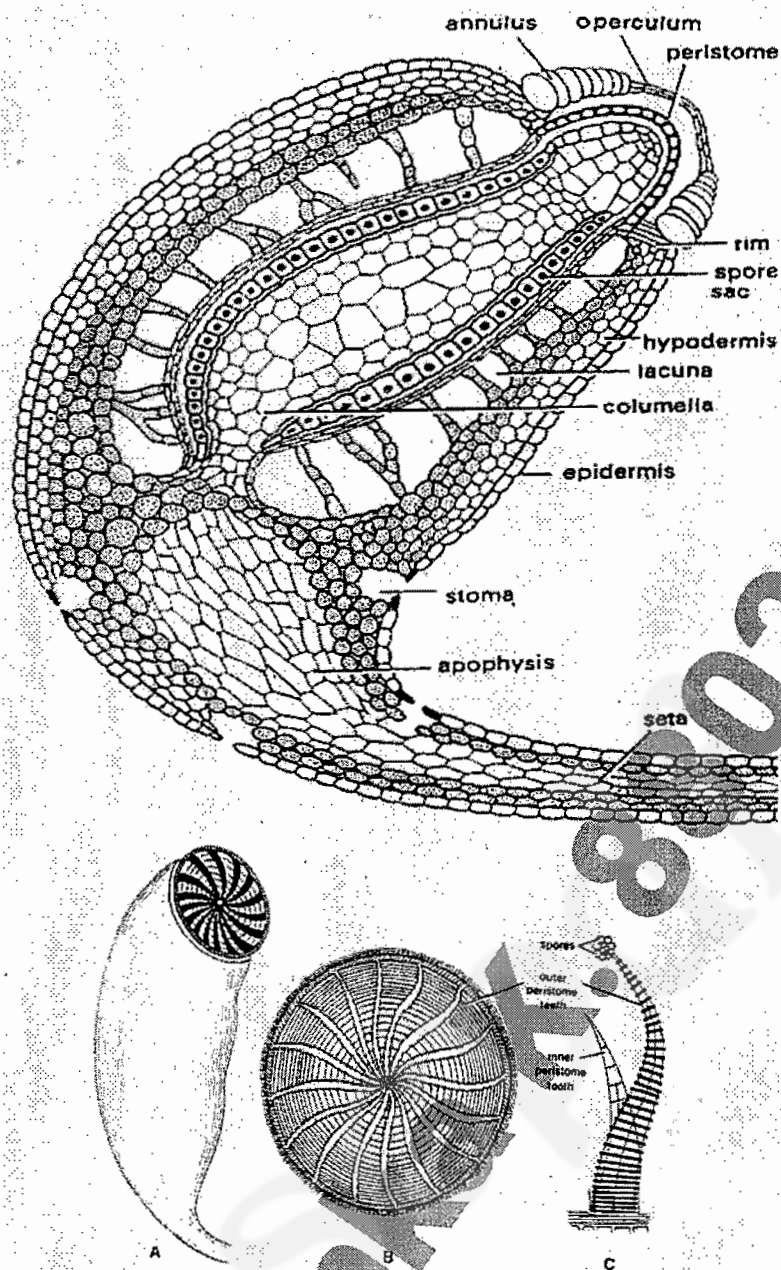


Figure 1: Sporangium in *Funaria*. A. Anatomy B. Operculum C. and D. Peristome teeth

structure between the foot and the capsule. The length of the seta varies in different species. Anatomically, the seta is differentiated into a single layered epidermis, a sclerenchymatous hypodermis, a parenchymatous cortex and a central cylinder. The central cylinder is composed of compactly arranged thin walled hydroid cells. The main function of the seta is to raise the capsule to the required height and conduction of water and nutrients.

3. **Capsule:** The capsule is differentiated into the following three parts.

- a. **Apophysis.** A bulbous apophysis is found at the base of the capsule. This is a sterile part. It is marked off from the fertile theca of the capsule by a distinct constriction. The apophysis has a thick walled epidermis. The epidermis is interrupted by stomata; each stoma has two guard cells. The epidermis is followed by a broad chlorenchymatous cortex with intercellular spaces. It serves as photosynthetic tissue. The central part of the apophysis is occupied by a conducting strand which is in continuation with columella and seta.

As the sporophyte matures, the water supply to the capsule is cut off. As a result, all tissues of the capsule, except spores, dry up. The thin walled cells of the annulus break and the operculum is thrown away.

The spores, however, are not dispersed after shedding of the operculum as the mouth of the theca is covered by peristome teeth.

The outer peristomial teeth are hygroscopic and in dry atmosphere they bend outwards with jerky movements, but the inner peristomial teeth remain in their position. Due to outward movements of the outer peristomial teeth, slits between the inner thin walled peristomial teeth become wider and spores escape through these slits gradually. In a wet atmosphere the outer peristomial teeth absorb moisture and bend inwards and thus closing the slits and prevent the escape of spores.

Thus, in *Funaria*, there is a built-in mechanism which allows the dispersal of spores only when the conditions for germination of spores are favourable.

Sporophyte in *Polytrichum*

Morphology and Anatomy

The sporophyte of *Polytrichum* is differentiated into a foot, a long seta and an angular capsule.

1. **Foot:** It is a dagger-shaped structure embedded in the female gametophore. It is composed of thin-walled parenchymatous cells. It acts as an anchoring and absorbing organ.

2. **Seta:** It is a long, slender

- b. **Theca.** It is the middle fertile part of the capsule. It is polygonal in outline due to the presence of many longitudinal grooves. The wall or jacket of the theca is composed of several layers of chlorophyllous cells. The outermost wall layer forms the epidermis which is devoid of stomata.

An air space or lacuna (**outer air space**) is present inner to the wall layers. It is divided into small chambers by transverse filaments of chlorophyll containing cells, the trabeculae.

One end of the trabeculae is connected with the inner wall layer and the other with the outer wall of the spore sac. The outer space is followed by **spore sac**, which is bounded on either side by a layer of thin walled cells. In later stages of development of the sporophyte spore mother cells form haploid spores by meiosis.

The spore sac is also surrounded on the inner side by an **inner air space** or lacuna. It is also traversed by many transverse trabeculae.

The central part of the capsule is occupied by a thick column of parenchymatous cells, the **columella**. This is a sterile tissue which is continuous with the central axis of the apophysis. The upper part of the columella is in contact with the **epiphragm**.

- c. **Operculum.** It is the apical part of the capsule which forms a cap-like structure at the apex of the theca. The base of the operculum is attached to the mouth of the theca. The boundaries of the operculum and theca are marked by a distinct constriction, the rim. In *Polytrichum*, annulus is absent.

The distal end of the columella is expanded into a pale thin walled membranous structure, the epiphragm. It is stretched like a drumhead (tympanum) across the capsule mouth.

In the mature capsule the peristome is composed of 32 or 64 peristomial teeth arising from the periphery of the diaphragm. Each tooth is a small, solid and pyramidal structure, made up of several layers of thick walled fibre-like cells. The peristomial teeth are directed upward and their base is fused with the margins of the epiphragm. Unlike *Funaria*, the peristomial teeth of *Polytrichum* are not hygroscopic.

Dehiscence of Capsule

At maturity the capsule shrivels as it dries up. The columella disintegrates and the spores come to lie in the cavity thus formed.

Further drying and shrivelling of the capsule wall causes the operculum to fall off and thus exposing the peristome.

At this stage the capsule is horizontally placed. After exposure of the peristome, thin walled cells of the epiphragm lying between the peristomial teeth also dry up. Thus several minute holes are formed in the margins of the epiphragm.

The spores are dispersed through these holes.

Only a few spores are liberated every time the capsule sways in the wind. This method of spore dispersal is known as censer mechanism.

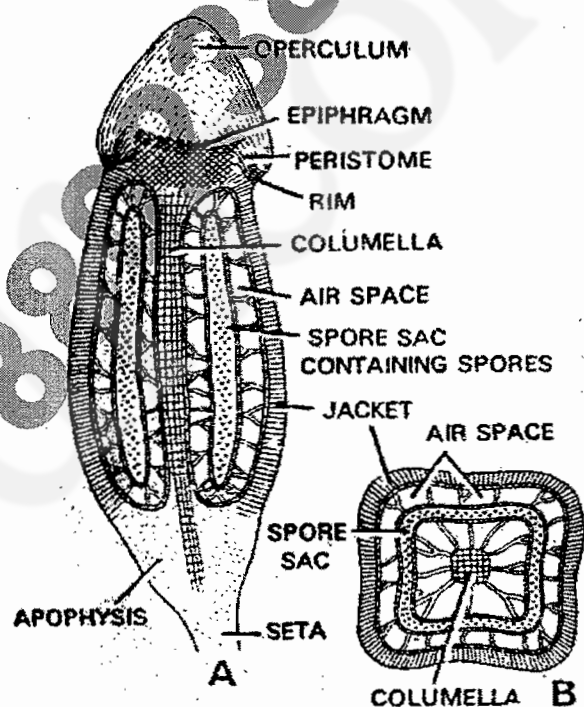


Figure 2: *Polytrichum* sporogonium. A. Longitudinal section. B. Transverse section

11. Sporophyte evolution in bryophytes

The life cycle of bryophytes universally displays heteromorphic alternation of generations. It means that the life cycle becomes complete by alternate appearance of a gametophyte and a sporophyte, which happen to be morphologically dissimilar with respect to one another. The alternation of generations in bryophytes is unique, in which the sporophyte is ecologically less persistent than the gametophyte. The sporophyte is physically attached to the gametophyte and shows at least a partial physiological dependency on the gametophyte as well.

A bryophytic sporophyte is a solid structure with a radial construction and it represents the diploid asexual generation in the life history. It is a product of diploid zygote and its chief function is production of spores through meiotic division and the dispersal of the spores by means of wind.

In morphology and internal organization the sporophyte varies from only a spherical spore producing case as in *Riccia* to an elaborately differentiated structure as seen in majority of the mosses.

Two theories have been put forward to explain the sporophyte evolution in Bryophytes.

1. **The theory of progressive sterilization or the progressive theory.** First proposed by Cavers (in 1912) and supported by Bowers (1935) and Campbell (1940).
2. **The theory of progressive simplifications or the regressive theory.** It was first proposed by Church in 1919 and supported by Prof. S. R. Kashyap, Evans and Geobel.

The Progressive Theory

This theory proposes two types of progression during the evolution of the bryophytic sporophyte.

1. Progression in anatomical complexity of the sporophyte; and
2. Progression in the amount of sterile tissue within the sporophyte.

According to the progression theory the most primitive sporophyte is seen in the family Ricciaceae and through various stages it culminates into the ultimate complexity of *Polytrichum* sporophyte. The various stages of sporophyte evolution proposed by this theory are as follows.

Riccia Stage: It represents the beginning material, showing and no foot or seta. Such a simple sporophyte probably arose from the ancestor *Sphaeroriccia* that had the gametophyte simplicity of *Sphaerocarpus* and sporogonial simplicity of *Riccia*. However, now most bryologists consider *Sphaeroriccia* a hypothetical plant.

In *Riccia* stage the reproductive allocation is maximum, where about 95% of the diploid cells within the capsule wall undergo sporogenic meiosis. The remaining 5% which fail to undergo meiosis may produce the nurse cells, providing nutrition to the maturing spores. But, the formation of the nurse cells is not a constant feature within the genus *Riccia* and it is confined only to certain species.

Corsinia Stage: The sporophyte in *Corsinia* has a sterile foot but no seta. The development of the foot enables the sporophyte to get firmly anchored into the gametophytic tissue, thus allowing for a better absorption of nutrition and water from the gametophyte. The sporogenous tissue is endothelial in origin. Reproductive allocation is about 85%. Thus, more cells function as nurse cells and nutritionally support sporogenesis.

Sphaerocarpus Stage: In *Sphaerocarpus*, apart from foot, a seta also develops. The seta is relatively slender but it at least it helps in pushing the spore capsule a bit higher. Such a location facilitates a wider spore dispersal and also enables the spore capsule to carry out photosynthesis. In addition to the seta formation, there are many more nurse cells (about 35%) in the spore capsule.

Targionia Stage: The seta in *Targionia* becomes massive. The foot also becomes enlarged. Thus morphological complexity increases greatly. Moreover, there are true elaters as well. The elaters are elongated and dead cells with spiral wall thickenings. They behave in a hygroscopic manner and aid in efficient spore dispersal.

Marchantia Stage: Foot and seta are as well developed as in *Targionia* stage but about 40% of the sporogenous tissue forms sterile elaters. Thus the level of sterilization rises sharply. In *Marchantia*, the seta elongates considerably just prior to the spore release.

Pellia Stage: The morphological differentiation is similar to the *Marchantia* stage, however the capsule wall becomes two layered and a cluster of elater called elaterophore develops. As a result, the anatomical complexity and the extent of sterilization rise. The elaterophores help in efficient spore release.

Anthoceros Stage: *Anthoceros* shows a sharp rise in sterilization of the sporophyte, although the morphology becomes poorly defined since the seta is absent. The reasons of increased sterilization are:

1. The wall of the capsule becomes multilayered.
2. Many potentially spore forming cells produce pseudoeolaters.
3. In the median region of the capsule, a massive columella with 16 rows of cells develops.

Moreover there are two obvious advancements in the sporogonium.

1. It grows perpetually due to a thin strip of meristematic cells at the juncture of the foot and the capsule.
2. The capsule wall shows photosynthetic activity. Thus showing a trend towards nutritional autonomy, although partially so.

Moss Stage: Considered to the ultimate derived stage where there is a sharp rise in the following three attributes.

1. Morphological differentiation.
2. Anatomical complexity
3. Extent of sterilization

Nearly, all the mosses except the members of Sphagnales show a well differentiated morphological plan of the sporogonium having foot, seta and capsule.

The capsule becomes differentiated into apothecial and thecal regions. The apophysis and some parts of theca shows photosynthetic activity and the presence of true stomata.

The theca is not completely dedicated to produce spores alone. It has a massive columella, multilayered wall, air columns with trabeculae and a distal operculum apparatus. All these structure divert above 80% of the diploid cells which could have been utilized for sporogenesis

Funaria and *Polytrichum* show ultimate anatomical sporogonial complexity. Despite a small reproductive investment the number of spores in the mosses is very high ranging from 10,000 to 200,000. Thus the members do not suffer in terms of proliferate potential.

Progressive simplification theory or regressive theory

In argument, this theory is exact opposite to the earlier theory. It considers the *Polytrichum* type sporogonium to be the most basal and *Riccia* type to be the most advanced or derived. The basis of such an argument is that increasing reproductive allocation must be treated as an advanced feature.

This theory proposes that during sporophyte evolution there has been a loss of morphological differentiation, anatomical complexity and the extent of sterilization. Evolution has favoured increasing reproductive allocation in the bryophytic sporophyte, according to this theory. In terms of percentage diploid tissue allocation for sporogenic purposes *Polytrichum* or other mosses show minimal allocation while *Riccia* shows the highest allocation.

This theory can be illustrated using the same examples as above but by constructing a series in the reverse order.

In terms of reproductive success, the theory of progressive sterilization appears to be more acceptable because:

1. Sterilization is increasing the amount of specialization and nutrition available to sporogenic cell enabling them to undergo more divisions and produce larger number of spores. It is a well established fact that despite minimal reproductive allocation the members of Polytrichaceae produce the highest number of spores per sporangia.
2. Sterile cells also add to functional specialization such as better conduction through columella, better gaseous exchange through stomata etc.
3. Due to functional specialization the sporogonium becomes partially independent in terms of nutrition.
4. Sterile cells ensure the best possible spore release mechanism and also the most appropriate timing for it. The examples include:
 - The explosive mechanism in *Sphagnum*.
 - The twisting and swaying mechanism of seta in *Funaria*
 - The hygroscopic movement of peristome teeth in *Funaria*.
 - The gate like function by the peristome teeth in *Polytrichum*.

Conclusion

Based in molecular phylogenetic studies by Kenrik and Crane (1997) and also by Linda Graham (2002), it is now established that the three major groups of Bryophytes have arisen parallel as sister groups from a single ancestral Cheatophoralean plexus. There can be no gradualistic linear relation among the sister groups. Thus our current understanding of Bryophyte evolution demands a re-examination into the issue of sporophyte evolution.

12. Spore dispersal in bryophytes

In bryophytes, the sporophyte generation is short-lived. It comprises a capsule which produces spores by meiosis and a stalk which holds this aloft the gametophyte. The spores once released are dispersed by air currents and, once they settle somewhere moist, germinate. This recommences the gametophyte generation.

Spore dispersal is an important aspect of bryophytes' functions. Once spores have been produced they need to be released and dispersed. There is considerable variation in sporophyte anatomy – in both the spore capsule and, when present, the supporting seta. All aspects of sporophyte structure have some influence on how the spores get out and are dispersed.

Most bryophytes rely on wind for spore dispersal. The vast majority of species have small spores, typically with diameters of 5 to 50 micrometres. Small spores can be carried considerable distances by the wind. Even very light breezes, virtually imperceptible to a person, can easily waft the smaller spores away.

Besides, there are certain other mechanisms available too. Wind dispersal gets more difficult with spores of about 50 micrometre diameter such as *Archidium* spores, which are too heavy for wind to be an effective dispersal agent. They are carried more easily by water. In addition, such spores may also be dispersed when mixed up with mud that is picked up by animal feet. There is also a small number of moss species in which insects are the main agents of spore dispersal.

Spore release and dispersal in liverworts

Release of the spores

In liverworts, two methods are found for spore release.

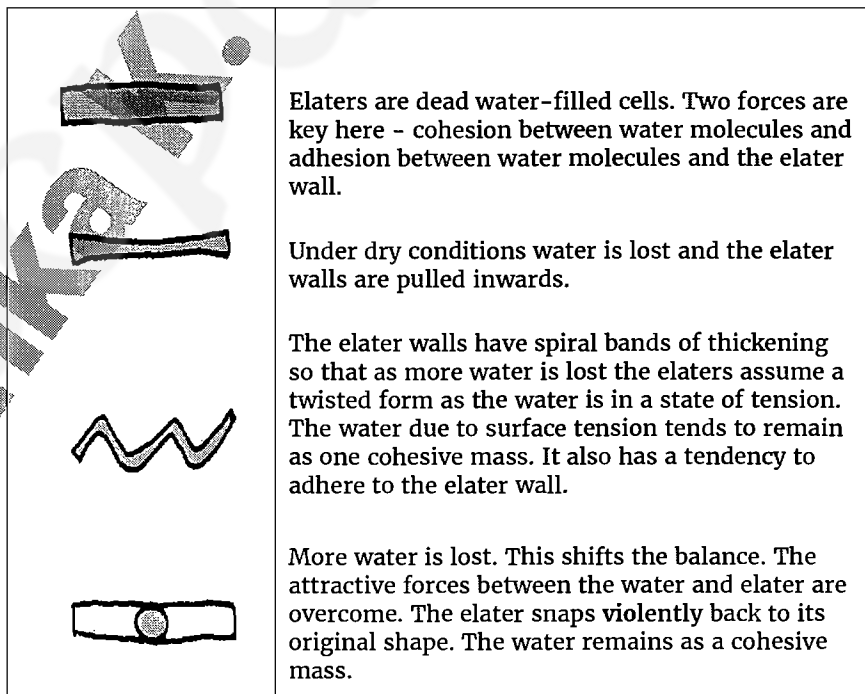
Sporangial degradation: In the complex thallose liverwort genus *Riccia* the spore capsules are embedded in the thallus. When mature the capsule and overlying thallus disintegrate, leaving the spores exposed within a cup-like depression. In *Fossombronia*, a simple thallose liverwort genus, a mature spore capsule is raised on a flimsy, translucent seta and the capsule wall breaks irregularly into small platelets, which fall away to expose the spore mass.

Splitting capsules: In most liverworts, the capsule splits into 4 valves. Usually there are four dehiscence lines and hence four arms in the open capsule. Within the capsules there are elaters as well as spores. Elaters are tubular cells with spiral thickenings that help in spore release. Elaters do not work in the same way in all species. The elaters may twist or untwist with changes in humidity, or spring suddenly when released from tension. In such cases the movement of the elaters helps fling the spores a short distance into the air where air currents can pick them up and carry them away.

Dispersal of the spores

Liverworts use both water and air for spore dispersal.

Water based dispersal: The spores in *Riccia* are commonly 60–80 micrometres in diameter and too large to be easily wind-dispersed, but water can wash them away. Moreover, as the *Riccia* thallus keeps growing at its tip, the older parts progressively disintegrate. So eventually any spores that have been unable to disperse from cup-like depressions in the thallus will be left loose on the soil, where they may germinate or disperse more easily.



Air based dispersal: Most bryophytes use wind as a mode of dispersal. Liverwort dehiscence and spore dispersal are timed to occur when there would normally be strong, drying winds to dry the outer layer of the capsule wall, causing the valves to curl backward. Since outer walls would dry first, they would be more contracted than inner walls.

Liverworts are aided in spore dispersal by elongate structures with spiral thickenings called elaters. These respond to changes in moisture, causing walls of cells between spirals to contract, thus resulting in twisting of elaters and contortion or bending of cells. When the elater reaches a certain point of tension due to remaining water adhering to walls of drying cells, it suddenly releases the remaining water and jerks into its original shape, thrusting nearby spores into the air.

Spore release and dispersal in mosses


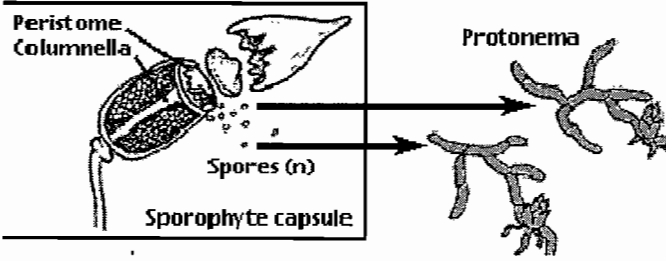
Release of the spores

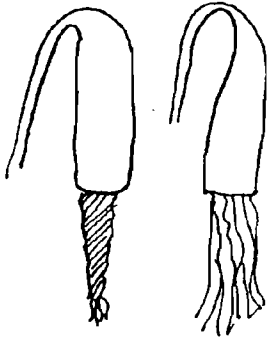

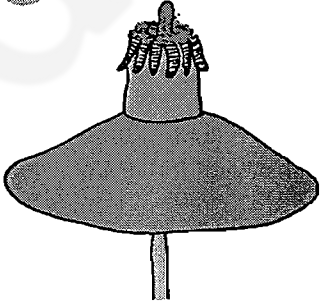
In mosses, with the exception of one group of mosses (Andreaeaceae or the Graphite mosses), all moss capsules have an operculum and peristome. In *Sphagnum* species, operculum is present, but peristome teeth are absent.

The general dispersal mechanism is as follows. Under dry conditions:

1. The calyptra (remnant of the archegonium) drops off
2. The operculum is shed as a result of water loss by the annulus
3. The peristome teeth bend outwards. (The peristome teeth are triangular two-ply structures which operate like trap-doors. One layer tends to readily absorb or lose moisture while the other has little affinity for water. What this means is that as water is lost one side of the peristome teeth shrinks while the other does not. This results in a bending of the teeth outward.)
4. Spores fall out of the capsule and are carried by air currents

The release mechanism in mosses, while showing general agreement to the plan outlined above, has certain genus specific uniqueness. These unique features are summarized below.

<p>Funaria Tips of the curved peristome teeth fuse in a central disc. Wet Teeth elongate and slits between teeth disappear. Dry Teeth shrink and gaps develop between teeth, allowing spores to sift out.</p>	
<p>Polytrichum The central disc or epiphragm is here very large and the peristome teeth are tiny. This peristome does not respond to moisture. Wet Epiphragm loose, teeth not stretched, gaps between teeth disappear. Dry Epiphragm taut, teeth stretched, gaps develop between teeth, allowing spores to sift out.</p>	

<p>Tortula Peristome teeth long and hair-like. Wet Teeth elongate and are tightly wrapped around each other. Dry Teeth shrink, disentangling, allowing spores to sift out.</p>	
<p>Sphagnum In <i>Sphagnum</i> the process is typically explosive, with spores and operculum shot off simultaneously. As the mature capsule begins to dry it shrinks, compressing the air inside. Eventually the internal pressure becomes enough to force the operculum off and shoot the spores into the air where breezes will pick them up.</p>	
<p>Splachnum This grows on dung and the capsule with its broad, skirt-like apophysis resembles a flower. The peristome forms a fringe at the top of the capsule. The columella sticks out of the top, covered in sticky spores. Flies are attracted to the capsule by its smell. The spores stick to the flies' feet and are soon dispersed.</p>	

Spore Release in *Andreaea*: In *Andreaea*, the capsule dehisces along vertical lines but the valves remain connected at their apex. As the atmospheric moisture decreases, the exothecial cells lose their water content and the valves arch outward, thereby exposing the spores. As the humidity increases, the cells swell and a reverse movement occurs. This closes the sporangium and protects the spore mass from water, which would trigger the premature germination of spores, but also agglutinate the spores and inhibit their effective dispersal by wind.

Dispersal of the spores

Majority of the mosses use air for spore dispersal. Mosses are aided in spore dispersal by elongate structures with spiral thickenings called peristome teeth. They are present around the operculum. These respond to changes in moisture.

Moss dehiscence and spore dispersal are timed to occur when there would be strong and drying winds.

Spore dispersal in hornworts

When the hornwort sporophyte is mature, it has a multicellular outer layer, a central rod-like columella. The pseudo-elaters are also present, which are multi-cellular. They have helical thickenings that change shape in response to drying out; they twist and thereby help to disperse the spores. Hornwort spores are relatively large for bryophytes, measuring between 30 and 80 μm in diameter or more.

The sporangial capsule open bivalvularly through slits and spores are mostly dispersed by winds and animals.

13. Economic and ecological importance of bryophytes

There are about 2500 species in Bryophytes found almost in all planes of the world. Most of the bryophytes are indirectly useful to man, however they play an important role in the economy of nature and management of ecosystem through being rock builders.

Economic importance

Bryophytes used as medicines

Number of Bryophytes are used as medicines in homeopathy. Chinese medicines include 40 different kinds of Bryophytes that have been used to treat diseases of the cardiovascular system, tonsillitis, tympanitis, cystitis and bronchitis and to cure skin disease and burns.

Marchantia polymorpha: This is a liverwort which is used to cure pulmonary tuberculosis (Roigy Mera, 1945). It is also used for liver treatments. *M. polymorpha* has antitumour properties thus, its extract is used to cure tumor. It is also used as medicine for boil and abscesses.

Marchantia stellata: This is also used in tumor ailments. Watt had mentioned the medicinal uses of *Marchantia polymorpha*, *Fagetellaconcia*, species of *Jungermanniales*, *Anthoceros* and *Riccia*. These are used as an external application to cure ringworm. The Chinese and the native Americans have used Bryophytes for example *Mnium* sp; and *Philontis* in the form of paste to cure wounds.

In India the burnt ash of mosses along with little oil and honey is used as an ointment for burns, cuts and wounds in the Himalayan region. The extract of *Rhodobryum giganteum* can increase blood circulation in aorta upto 30% in animals.

The species of *Sphagnum* found in temperate countries is widely used for medicinal purposes. A healing ointment prepared by *Sphagnum* leaves mixed with grease is used in the treatment of cuts and wounds. The decoction prepared by boiling dried *Sphagnum* in water, is used in the treatment of acute haemorrhage. This decoction is also used to cure diseases of eyes.

The lakes where *Sphagnum* grows are called as *Sphagnum* bogs. These are well known to produce peat in acidic water. The bog water is antiseptic and has astringent properties.

Because of its absorbent and antiseptic properties, *Sphagnum* was used for dressings during World War I, to make pillows for resting of wounded members for soldiers transported to hospitals from battlefields, and recently as filling material for sanitary napkins.

Antibiotics from Bryophytes: The Bryophytes are delicate land plants and are devoid of thick cuticle and bark therefore they have biochemically active compounds which perform defense mechanism to protect them from enemies like fungi, bacteria and insects. Some liverworts are known to have lunularic acid for example in the extracts of *Reboulia* and *Pallavicinia*. It shows antimicrobial properties. Due to antimicrobial activity liverworts are not susceptible to fungal disease. Lunularic acid inhibits the growth of pathogenic fungi *Botrytis cinerea*, *Rhizoctonia solani* and *Pythium* spp. whereas petroleum ether extracts of *Barbula* and *Timmiella* species were found to be active against both gram negative and gram positive bacteria.

A number of unsaturated lipids, fatty acids, esters flavonoids, tripenoids and phenols have been reported from Bryophytes. Hayes reported that aqueous extract of *Conocephalum conicum* has antibiotic activity. It is found to contain Norpiguison. Bryophytes *Polytrichum* and *Sphagnum* exhibit strong antibacterial properties against *Gaffkeya tetragena* and *Staphylococcus aureus*.

Bryophytes as an experimental material in Lab

Bryophytes, both liverworts and mosses are utilized as an important tool for research in lab. It is used as a test material in a number of branches of botany such as ecology, experimental morphology, toxicology, genetics, physiology, reproduction biology, biochemistry and pharmacognosy. During green house experimental plants like tomato, pepper, cucumber and wheat treated with liverwort extract were found to be less infected with fungus infection by *Phytophthora infestans* than unrelated plants.

Bryophytes used as Food Material

Bryophytes are directly used as food material by those animals that are useful to man. *Polytrichum* and *Bryum* are used to make capsular food as a main diet of the chicks, Besides some birds like field fare, song

thrush and black bird use mosses as a regular food. Reindeer depends on *Polytrichum* and *Hylocomium alaskamum* for food.

Use of Bryophytes as fuel

Liverworts and mosses have long been tried and utilized as a fuel in developed countries like West Germany, Sweden, Finland, Poland and Soviet Union. *Sphagnum* peat is suitable for production of low and intermediate BTU gas as well as hydrogen, ethylene, natural gas, methanol and gasoline. Peat moss is most suitable for the production of methane. Peat is likely to become an important future source of fuel for production of heat and electricity besides methane as its heating value is superior to that of wood because of low sulphur content. The peat moss harvesting, processing, and sale of *Sphagnum* peat is now a multimillion-dollar industry.

Use of Bryophytes in Horticulture

Bryophytes provide good absorbing material, hence are of great use in horticulture. They improve soil quality, moisture content and increase mineral nutrients in the field if added to dry soil. They may be added to pots to hold moisture and mineral contents. They are extensively used for various purposes by the horticulturists.

In Japan, *Sphagnum* has traditionally been used as base cover for bonsai, in miniature landscapes and in the designs of the famous Japanese gardens.

Use of peat moss in Waste Water Treatment

The peat is highly absorbent and permeable physically. It is known to absorb toxic metals, therefore, *Sphagnum* is used as an effective filtering and absorption agent for the treatment of waste water and effluent of factories with acid and toxic discharge consisting of heavy metals particularly Ag, Pb, Cu, Hg, Fe, Sb and organic substances like detergents, dyes, microorganisms and soils.

Packing material

Sphagnum or peat moss has some economic importance as packing material for breakable or fragile objects such as figurines and dinnerware's. It is also used as packing materials for transporting plants and plant parts, since *Sphagnum* holds water and hence prevent plants from drying during transport.

Ecological importance

Bryophytes as an indicator of Environmental Conditions

Bryophytes are good indicators of environmental conditions. Mosses can be used as an indicator of calcium and nutrient content in water. Some Bryophytes can grow only in narrow and specific pH range and therefore their presence can be treated as an indicator of soil pH. *Mercuria* and *Michiofera elongata* are indicators of the presence of copper rich soil. Such plants are used as indicator plants for copper rich soil.

Mosses do not possess a protective epidermis and thick cuticle to protect themselves, therefore they are good indicators of acid rain. In polluted areas, standard transplantation of certain mosses has been found to be quite useful for monitoring the intensity and trend of air pollution.

Bryophytes Prevent Soil Erosion

Mosses like *Pogonatum*, *Nardia* and *Blasia* play a key role as inhibitors of soil erosion, Moss like as *Dicranum* and *Rhodobryum* prevent soil erosion on the slopes of hills. Besides, they provide excellent bed for seed germination.

Rock Builders

Mosses like *Bryum* and *Hymen* grow in water bodies rich in calcium and bicarbonate in association with aquatic plants perform a key role in rock builders. The bicarbonic ions are converted into the insoluble calcium carbonate by the plants which get precipitated and hardened forming calcareous rocks like deposition. These rock deposits can be used as building material.

Soil Formation and Preparation of Substratum Layer for Vegetation

Bryophytes play an important role in soil formation. They germinate and grow in the cracks of the rock. After their death and decay, they add organic matter to almost sterile soil to make them fertile. Thus, they prepare suitable substratum with accumulation of organic matter for other plants to grow. Thus Bryophytes are pioneer plants for lithosphere i.e. succession on rocks.

Ecosystem stabilization

Bryophytes are very important in initiating soil formation on barren terrain, in maintaining soil moisture, and in recycling nutrients in forest vegetation.

In the tropical rainforest, 'moss balls' form in the higher elevations. Here they can absorb great quantities of rain and release water slowly into the atmosphere or ground. They also, along with the moisture, release quantities of ions i.e. Ca^{+} . These balls support numbers of invertebrates and smaller organisms.

In wetlands, bryophytes absorb great quantities of water and release organic acids which decrease decomposition rates. This accumulation of biomass over thousands of years forms ecosystems such as Swamps of various types.

PART – VI

PTERIDOPHYTA

Overview of the Pteridophytes

Pteridophytes and their salient features

The term **Pteridophyte** to refer to non-seed vascular plants. This group has also been referred to as vascular cryptogam and classified by Carolus Linneaus (1754) under the class Cryptogams.

The group has a long fossil history. They arose in the Silurian. *Cooksonia caledonica*, a fossil species, is so far oldest known vascular plant.

Salient Features of the Pteridophytes

Habitat

1. The pteridophytes grow under varied habitats. Most of them are terrestrial plants and thrive well under damp and shady conditions, while some flourish well in open grasslands, exposed dry places or even under xeric conditions (e.g., *Selaginella lepidophylla*, *S. pilifera*, *Gleichenia* etc.).
2. A few pteridophytes are aquatic or semi-aquatic (e.g., *Marsilea*, *Azolla* and *Salvinia*).
3. A few species may be epiphytes (e.g., *Lycopodium phlegmaria*, *Psilotum flaccidum*, *Selaginella 348terido*, *Ophioglossum pendulum* etc.).

Sporophytic Plant Body

1. The main independent plant body of Pteridophytes is sporophyte. It develops from the diploid zygote. It displays great variation in form, size and structure. Most of the present day 348teridophytes are herbaceous except a few woody tree ferns (*Cyathea spinulosa*, *Diksonia*, etc.).
2. Plants are differentiated into true roots, true leaves and true stems. However, in case of the extinct group Rhyniophytes (e.g. *Rhynia*) and living genus *Psilotum*, the leaves and roots are absent. Their functions are taken up by the other organs. For example, the roots are replaced by rhizoids and the stem axis becomes photosynthetic.
3. Plants mostly exhibit radial symmetry or they can rarely be dorsiventral.
4. The sporophyte has well developed vascular system. The vascular system is arranged in various types of steles in different groups.
5. The branching of stem may be dichotomous or monopodial.
6. Secondary growth does not occur in most of the living pteridophytes (except in *Isoetes*, *Cyathea*, and *Diksonia*).

Reproduction

1. The sporophytic plant body produces haploid spores, which serve as means the asexual reproduction.
2. The spores are produced inside the sporangia, which may be borne on stem or leaves. The leaves bearing sporangia are called sporophylls.
3. The development of sporangia may be eusporangiate, i.e., the sporangium originates from a group of initial cells (e.g., *Psilotum*, *Lycopodium*, *Selaginella*, *Equisetum*, etc.) or leptosporangiate, i.e., the sporangium originates from a single superficial cells (e.g., *Marsilea*, *Pteridium*, *Pteris*, etc.).
4. The sporophylls may be scattered on the plant or may be restricted to particular regions called strobili or cones. In some cases, the sporangia are produced within pteridophytes structures called sporocarps (e.g., *Marsilea*).
5. In some higher pteridophytes, the sporangia are borne in small groups on the sporophyll. Each group is called **sorus**.
6. It may be protected by false indusium (e.g., *Pteris*), true indusium or both (e.g., *Pteridium*).
7. The plants may be homosporous, i.e., produce only one type of spores (e.g., *Psilotum*, *Lycopodium*, *Equisetum*, *Pteridium*, *Pteris*, etc.) or heterosporous i.e., produce two different types of spores—smaller microspores and larger megaspores (e.g., *Selaginella*, *Marsilea*, etc.).

Gametophyte

1. The sexual reproduction is oogamous. The male sex organs are antheridia and female sex-organs are archegonia.
2. The spores germinate to produce haploid gametophytic plant bodies, known as **prothallus**. Homosporous pteridophytes produce monoecious prothalli, i.e., both antheridia and archegonia are

borne on the same prothallus. The heterosporous pteridophytes produce dioecious prothalli, i.e., antheridia are borne on male prothallus and archegonia are borne on female prothallus.

3. The gametophytes may be exosporic or endosporic.
4. The antheridia are either embedded in the tissue of gametophyte (e.g., *Lycopodium*, *Equisetum*) or projected above the surface (e.g., *Pteris*, *Pteridium*). Each antheridium has single layered sterile jacket enclosing a large number of androcytes. The androcytes metamorphose into flagellated motile antherozoids.
5. The archegonia typically resemble those found in bryophytes. Each archegonium is flask shaped, embedded in the tissue of gametophyte. It is differentiated into neck and venter. The neck consists of four vertical rows of cells whose height varies from two to six cells. The axial rows consist of neck canal cells, venter canal cell and an egg. The neck canal cells vary from one to 14 in number.
6. Fertilization occurs in presence of water. It is needed for dehiscence of antheridia, liberation of antherozoids, movement of antherozoids from antheridia to archegonia, maturation of archegonia and syngamy.
7. The haploid antherozoid fuses with the haploid egg and form diploid zygote, which is the first cell of sporophytic generation.

The Embryo (Young Sporophyte)

1. The diploid zygote is retained within the archegonial venter. It develops into a young sporophyte (the embryo), which remains attached to the gametophyte and gets nourishment during its early stages from gametophyte.
2. The development of embryo may be endoscopic, i.e., the axis of embryo directed inwards away from archegonial neck (e.g., *Lycopodium*, *Selaginella*) or exoscopic, i.e. the axis of embryo directed outwards towards archegonial neck (e.g., *Psilotum*, *Equisetum*). In some cases, it is lateral, i.e., the axis of embryo is at right angles to the long axis of archegonium (e.g., *Marsilea*, *Pteridium*, and *Pteris*).
3. The alternation of generation, in all the members of pteridophytes, is heterologous type (heteromorphic).

Life Cycle of Pteridophytes

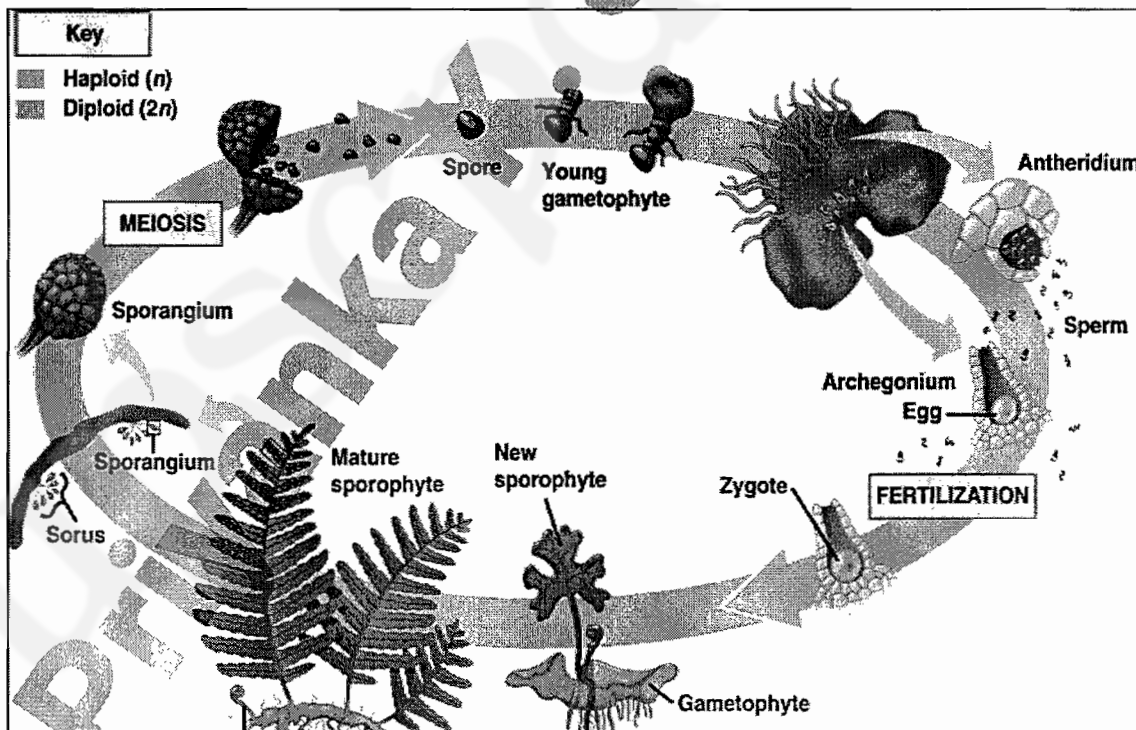


Figure1: The life cycle of Pteridophytes, displaying Alternation of Generations

The life of pteridophytes comprises of two morphologically distinct phases, as shown in Figure 1.

1. The gametophytic phase and
2. The sporophytic phase (or sporophytic generation).

It is a general rule that a gametophytic plant body is followed by the formation of sporophytic plant body and the sporophytic plant body is followed by gametophytic plant body. In other words, the two generations alternate with each other.

The Gametophyte

The gametophyte (also called prothallus) is haploid. It is concerned with the sexual reproduction.

It produces male sex-organs-antheridia and female sex-organs-archegonia. The gametophytic prothalli of homosporous pteridophytes (e.g. *Lycopodium*, *Psilotum*, *Pteridium*, *Pteris* etc.) are small, inconspicuous, autotrophic or heterotrophic and bisexual (monoecious). They bear both the sex-organs in the same plant.

The gametophytes of heterosporous pteridophytes (e.g., *Selaginella*, *Marsilea*, etc.) are highly reduced, usually endosporic and unisexual (dioecious), i.e., the male gametophytes, produced from microspores, bear only antheridia and the female gametophytes, produced from megaspores, bear only archegonia.

The structure of sex-organs is essentially similar to that of bryophytes. The antheridia may be embedded or projecting type. Each antheridium has single layered sterile jacket enclosing a mass of androcytes. The androcytes metamorphose into flagellated motile antherozoids (sperms). They liberate from mature antheridium and swim in water to reach archegonia.

The archegonia are usually flask shaped, shortly stalked or sessile and differentiated into globular venter and tubular neck. It consists of sterile jacket enclosing a variable number of neck canal coils, single ventral canal cell and a large egg. The egg is non-motile and thus, the mode of sexual reproduction is oogamous.

Fertilization (syngamy) occurs in presence of water. The haploid antherozoid fuses with the haploid egg and produces diploid zygote. The gametophytic phase of life cycle ends with the production of zygote.

The sporophyte

The diploid zygote is the first cell of sporophytic generation. It is retained within the archegonial venter where it germinates to produce an embryo (young sporophyte). The embryo development may be exoscopic (e.g., *Psilotum*, *Equisetum*, etc.) or endoscopic (e.g., *Lycopodium*, *Selaginella*, etc.). In some cases, embryo development is lateral i.e., its axis is at right angles to the long axis of archegonium (e.g., *Marsilea*, *Pteridium* etc.). The young sporophyte, later establishes as independent plant. It develops anchoring organs (root) and photosynthetic shoot.

The sporophytic phase of life cycle is dominant and long-lived. The plants are usually differentiated into root, stem and leaves. They are morphologically more complex. The plants develop vascular tissues (i.e., xylem and phloem). At maturity, they bear sporangia either aggregated in specialized structures called strobili (cones) or sporocarps.

Each sporangium encloses spore mother cells, which divide by meiosis and produce haploid spores.

Each spore mother cell gives rise to four haploid spores, which are usually arranged in tetrads.

The spores of homosporous pteridophytes are alike, but those of heterosporous pteridophytes are of two types – the microspores and megaspores.

The sporophytic generation ends with the production of spores. Each spore is the pioneer cell of gametophytic generation. It germinates to produce gametophyte.

Thus, the two generations of life cycle the gametophytic generation and the sporophytic generation, alternate with each other. It is called alternation of generations. Since the two generations exhibit marked morphological and anatomical differences, the alternation of generations is heteromorphic (or heterologous) type.

The Current Grouping of the Pteridophytes

Traditionally, the term Pteridophytes has been treated synonymously to seedless vascular plants. However, it is now recognized that the seedless vascular plants do not make one monophyletic group. Therefore, there are **six distinct phyla** recognized for the seedless vascular plants. Of these, **4 phyla** are now extinct and **2 phyla** have extant members. **The 2 phyla with extant members are listed in Table-1 below** (after Smith, 2006; Simpson, 2007; Dickinson, 2008).

Phylum	Class	Important Orders
LYCOPODIOPHYTA	Lycopodiopsida	Lycopodiales
	Sellaginellopsida	<i>Selaginella</i> les
	Isoetopsida	Isoetales
PTERIDOPHYTA (Ferns and allies)	Equisetopsida	Equisetales
	Psilotopsida	Psilotales
		Ophioglossales
	Polypodiopsida	Marattiales
		Osmundales
		Hymenophyllales
		Gleicheniales
		Schizaeales
		Dicksoniales
		Cyatheaales
		Marsiliales
		Salviniales
		Pteridales
		Blechnales
		Polypodiales

In addition, there are 4 phyla of extinct seedless vascular plants. These extinct groups include:

1. Rhyniophyta
2. Zosterophyllophyta
3. Trimerophytophyta
4. Progymnosperms

A brief account of individual groups of extant seedless vascular plants

Lycopodiophyta: The Division Lycopodiophyta is the oldest extant (living) vascular plant division at around 420 million years old, and includes some of the most "primitive" extant species. Some members are homosporous while others are heterosporous. They differ from all other vascular plants in having microphylls, leaves that have only a single vascular trace (vein). The members of this division have a long evolutionary history. The Silurian species *Baragwanathia longifolia* represents the earliest identifiable Lycopodiophyta. The extant genera are *Lycopodium*, *Stylites*, *Lycopodiella*, *Phlegmarius*, *Huperzia*, *Selaginella*, *Isoetes* etc.

Equisetopsida: Living species are commonly known as horsetails. They have needle-like leaves radiating at regular intervals from stem. Their sporophytic bodies comprise photosynthesizing, segmented, hollow stems, sometimes filled with pith. At the node between each segment is a whorl of leaves. In the only extant genus *Equisetum*, these are small leaves (microphylls) with a singular vascular trace. However, sphenophyte leaves probably arose by the reduction of a megaphyll, as evidenced by early fossil forms such as *Sphenophyllum*, in which the leaves are broad with branching veins.

Psilotopsida: They are also called whisk ferns. They were once considered to be the most primitive among the vascular seedless plants, closer to the Rhyniophytes. However, it is now known through molecular phylogenetic studies that Psilotopsida is the sister-group to all other ferns (Pryer; Smith *et al*, 2004). Smith *et al*. (2006) classified this phylum with two orders Psilotales and Ophioglossales, respectively.

(Note: Ophioglossales are traditionally included in the division Pteridophyta, but recent molecular systematic studies have shown the Ophioglossales to be closely related to the Psilotales.)

Polypodiopsida: this is also known as the group of true ferns. It has about 20,000 species.

Important features of true ferns are as follows.

1. **Stems:** Most often an underground creeping rhizome, but sometimes an above-ground creeping stolon (e.g., Polypodiaceae), or an above-ground erect semi-woody trunk (e.g., Cyatheaaceae).

2. **Leaf:** The leaves are always megaphyllous and pinnately compounded. In ferns, the leaf is also referred to as a frond. New leaves expand by the unrolling of a tight spiral called a fiddlehead. This uncurling of the leaf is termed *circinate vernation*. Leaves are divided into two main types:
 - a. **Trophophyll:** A leaf that does not produce spores, but carries out photosynthesis.
 - b. **Sporophyll:** A leaf that produces spores.
3. **Roots:** The underground non-photosynthetic structures that take up water and nutrients from soil. They are always fibrous and are structurally very similar to the roots of seed plants.

Comparison of Pteridophytes with Bryophytes and Gymnosperms

The Pteridophytes occupy a unique position between the bryophytes on the one hand and the spermatophytes on the other.

Similarities with Bryophytes

1. Plants of both the groups are terrestrial in habit.
2. The sporophytes reproduce by production of haploid spores. The diploid spore mother cells divide by meiosis to form tetrads of spores.
3. The structure and ontogeny of sex-organs (*i.e.*, antheridia and archegonia) are similar in both the groups.
4. The antheridia have sterile jacket cells enclosing the mass of androcytes. Each androcyte metamorphoses into flagellated motile antherozoid.
5. The archegonia have sterile protective covering around the neck canal cells, ventral canal cell and egg.
6. Presence of water is essential for dehiscence of antheridia, opening of archegonia and fertilization.
7. The fertilized egg (zygote) is retained within the archegonium and develops embryo.
8. Young sporophyte is dependent on gametophyte for nourishment.
9. Plants of both the groups show a distinct heteromorphic alternation of generations.

Differences between Pteridophytes and Bryophytes

Plants of pteridophytes differ from bryophytes in the following features:

1. The dominant phase of life cycle, in pteridophytes, is sporophyte, whereas in bryophytes the dominant phase is gametophyte.
2. The sporophytic plant body is differentiated into root, stem and leaves.
3. All the vegetative parts of sporophyte possess vascular tissues (*i.e.*, xylem and phloem).

Similarities with Gymnosperms

1. The dominant phase of life cycle is sporophyte. The gametophytes in both the groups are small and inconspicuous.
2. The sporophytic plant body is differentiated into root, stem and leaves.
3. The sporophyte shows elaborate tissue differentiation.
4. All the vegetative parts of sporophytes, in both the groups, have vascular tissues (*i.e.*, xylem consisting of tracheids and xylem parenchyma and phloem consists of sieve-tubes and phloem parenchyma).
5. The process of photosynthesis is usually restricted to special organs *viz.*, leaves. The leaves are provided with stomata.
6. The plants show distinct heteromorphic alternation of generations.

Differences between Pteridophytes and Gymnosperms

1. Nearly all gymnosperms are trees or shrubs, while almost all the Pteridophytes are herbaceous plants.
2. The Pteridophytes show primitive type of stelar construction (Protostele) in many cases, which are not seen in Gymnosperms.
3. The gymnosperms are seed producing plants, while the Pteridophytes produce spores to disperse.
4. The gymnosperms are always heterosporous, while the Pteridophytes show heterospory in a limited number of genera.

5. The male gametophyte in gymnosperm is a tubular structure known as pollen tube. Such structure is totally absent in all Pteridophytes.
6. Most modern gymnosperms have non-motile male gamete, which is not at all seen in the Pteridophytes.

Stelar System

Stelar System in Pteridophytes

The central vascular cylinder of the primary axis of plants is known as **STELE** (Greek. Stele=column). Besides xylem and phloem, it includes pith (if present) and is delimited from the cortex by the pericycle.

The concept that stele is the fundamental unit of vascular system was put forward by van Tieghem and Douliot (1886). They proposed the stelar theory.

Types of stele in Pteridophytes

Schmidt (1982), Eichorn and McElwain (2007) have recognised the two principal types of stele in Pteridophytes.

1. Protostele
2. Siphonostele

Protostele

It is a non-medullated (lacking pith) stele consisting of a central core of xylem, surrounded by a band of phloem. There is a single or multiple layer of pericycle outside the phloem.

The protostele is considered to be the most primitive, both phylogenetically as well as ontogenetically.

Fossil Psilophytales (e.g. – *Rhynia*, *Horneophyton*), as well as many living members (e.g. – *Psilotum*, *Tmesipteris*, most species of *Lycopodium*), characteristically show this type of stele.

The protostele gives off leaf traces without any break in the continuity of the enveloping endodermis. With the departure of leaf trace there is no marked change in the solid xylem core. Each leaf trace is surrounded by its own endodermis (as seen in *Lycopodium* or in *Tmesipteris*).

4 types of protostele are recognised in Pteridophytes (Refer to and insert the class lecture figures).

Haplostele– (a) Simplest and most primitive type of protostele. (b) Consists of a solid xylem core with smooth circular outline, which is surrounded by a ring of phloem. (c) Found in fossil (e.g. – *Rhynia*, *Horneophyton*, *Cooksonia*) as well as many living pteridophytes (e.g. – *Psilotum*, *Selaginella*, *Lycopodium*).

Actinostele–(a) Xylem is star-shaped with many radiating arms. (b) The phloem, instead of forming a continuous ring, is present in small patches in between the radiating arms of the xylem. (c) Characteristic of many living (e.g. – *Psilotum*, *Lycopodium serratum*) and fossil forms (e.g. – *Asteroxylon*, *Sphenophyllon*).

Plectostele–(a) Central xylem core breaks into more or less parallel plates. (b) Each xylem plate is surrounded by phloem. (c) Aerial shoots and cone axis of *Lycopodium clavatum*, and *L. volubile* have this type of stele.

Mixed protostele–(a) The solid xylem core is broken into small groups of tracheids which remain embedded in the phloem. (b) Found in stems of *Lycopodium cernuum*. (c) A mixed protostele is different from a protostele with mixed pith. The latter possesses groups of tracheids intermixed with parenchyma cells. (e.g. – *Osmunda regalis*).

Siphonostele

Siphonostele is a derived condition from the protostele. It contains a pith. Pith is a non-vascular tissue present in the centre of the vascular cylinder. Pith is composed mostly of thin walled parenchymatous cells. In some taxa, it may contain sclerenchyma cells also.

There are two types of siphonosteles on the basis of their association with leaf and branch traces.

1. **Cladosiphonic**: A cladosiphonic stele is characterised by the absence of leaf gaps or branch gaps. Mycophyllous lycopsids are the main example. (Refer to and insert the class lecture figures).
2. **Phyllosiphonic**: Phyllosiphonic stele has both leaf and branch traces with leaf and branch gaps. The leaf gap is a break in the vascular tissue of a stem above the point of attachment of a leaf trace. This gap is filled with parenchyma tissue. (Refer to and insert the class lecture figures). It is found in the members of the order Filicales.

Siphonosteles of *Equisetum*, *Osmunda*, etc., have a single phloem ring external to the xylem. Such a siphonostele is called ectophloic siphonostele. Contrary to this, the siphonosteles of *Adiantum*, *Dryopteris* and *Marsilea* have a ring of phloem each external and internal to the xylem. This type of siphonostele is called amphiphloic siphonostele. Such steles characteristically have two endodermal layers: outer

endodermis – that lies outside the outer phloem and inner endodermis – that lies inner to the inner phloem, outside the pith.

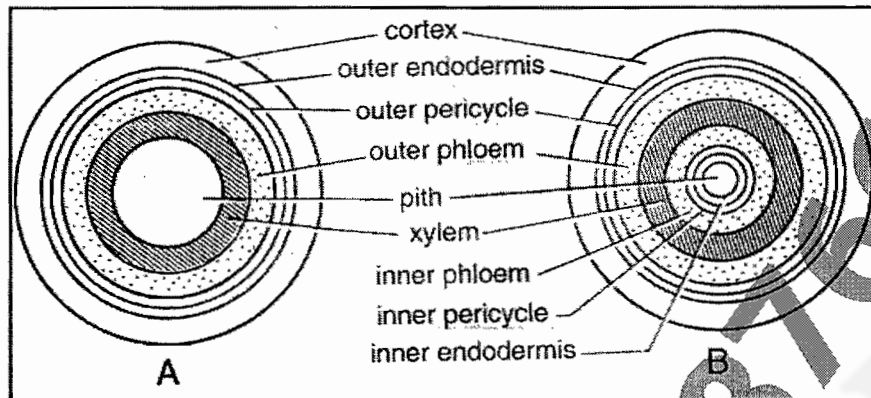


Figure 3: A. Ectophloic siphonostele; B. Amphiphloic siphonostele

Origin of Siphonostele–Siphonostele has originated by the development of pith in the centre of the protostele. The process of medullation of protostele has been explained by two views–

1. **Intra-stelar origin of pith**–According to this view, pith originated as a pro-tracheary elements of the central xylem core could not differentiate into proper tracheary elements and remained parenchymatous. Thus the pith is wholly intrastelar. Examples cited in support of this theory are *Botrychium ternatum*, *Osmunda regalis*, etc.– where are tracheids are scattered throughout the pith (i.e., mixed pith). In the light of recent evidences (McElwain, 2003), this theory is considered more acceptable than the Extra-stelar origin theory for the pith.
2. **Extra-stelar origin of pith**–According to this theory, the protostele has transformed into siphonostele due to the migration of cortical cells into the stellar axis through openings, such as leaf and branch gaps. This view derives support from amphiphloic siphonostele that has two endodermal layers. However, it is no longer considered valid because fossil evidences establish that the pith originated before the advent of leaf and branch gaps.

Modifications of Phyllosiphonic Siphonostele

Depending on the presence of non-overlapping or overlapping gaps, the following three types of siphonosteles are recognised –

1. **Solenostele**– (a) A siphonostele with non-overlapping leaf gaps. (b) The siphonostele may be ectophloic or amphiphloic. (c) Usually in the lower part of the stem where leaves are sparsely placed, the stele is solenostele. (Refer to and insert the class lecture figures).
2. **Dictyostele**–(a) Many ferns like *Dryopteris*, *Pteris*, *Ophioglossum*, etc., have a very small rhizome with crowded leaves. Consequently, the leaf gaps overlap with each other. A siphonostele with overlapping leaf gaps –Dictyostele. (b) A dictyostele has many scattered vascular strands, each of these strands is called a meristele. (Refer to and insert the class lecture figures).
3. **Polycyclic stele**– (a) Stellar structure of certain pteridophytes possess two or more concentric rings of vascular tissue–Polycyclic. (b) For instance, there are two rings of vascular tissue in *Pteridium aquilinum*, three in *Montania pectinata*, etc. (c) If in a polycyclic stele the outer cylinder is solenostelic, it is called polycyclic solenostele and if the outer cylinder is dictyostelic, it is known as polycyclic dictyostele. (Refer to and insert the figures below).

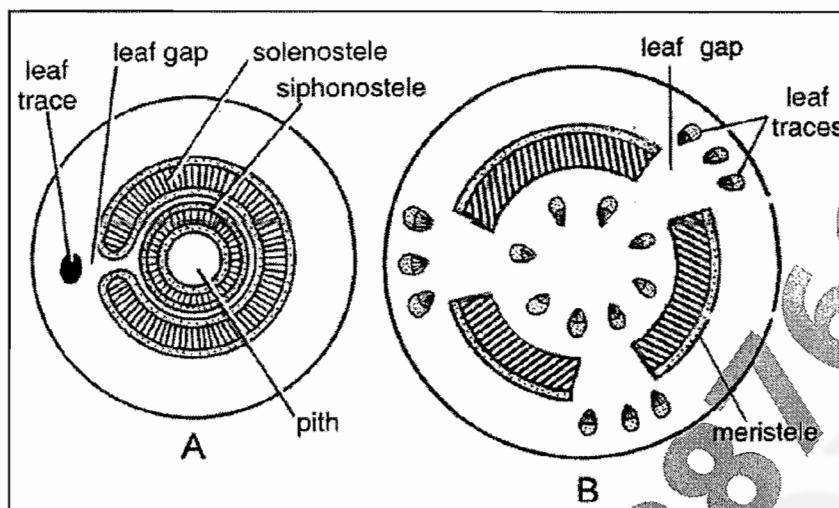


Figure 4: A. Polycyclic Solenostele; B. Polycyclic Dictyostele

Evolutionary relations among the different types of steles

In 1982, C. B. Beck *et al* concluded the following trends in the stelar evolution.

1. Protostele is the most primitive type, while siphonostele is the derived condition.
2. The haplostele is the most primitive type of protostele.
3. Haplostelic condition has given rise to actinostelic, plectostelic and mixed-protostelic conditions.
4. The origin of earliest siphonostele occurred from haplostelic ancestor by internal development of pith.
5. Cladosiphonic condition is primitive while Phyllosiphonic condition is advanced.
6. The earliest Phyllosiphonic condition was Solenostele. Thus, the unilacunar type (just one leaf gap) is primitive, multi-lacunar ones (Dictyostele) are regarded as derived.
7. Most of the fossil evidences support an origin of the eusteles from protostelic ancestors and not from siphonostelic ancestors. Eustele is the basic arrangement of the primary vasculature of seed plants. It is absent in pteridophytes. The early steps in the evolution of the eustele of the Calamopityaceae started with the medullation of three-ribbed protosteles characteristic of species of *Stenomylon*. Additional medullation, resulting from failure of centrally located metaxylem cells to differentiate, occurred in *Calamopitys Americana*. In *C. Americana*, the five protoxylem strands appear as discrete axial bundles from which leaf traces diverge on the radius of the stem. Further medullation resulted in the formation of a pith around which is a clearly defined ring of five axial bundles. This is apparent in *Calamopitys* sp.
8. Vascular systems with five traces are most likely primitive; those with more or less traces are derived.

Heterospory and Seed Habit

Heterospory in Pteridophytes

The production of two kinds of spores differing in structure and function, the smaller one producing the male gametophyte and larger one producing the female gametophyte, is termed **heterospory**. Heterospory has been of considerable interest because of its bearing on the evolution of seed.

In land plant evolution, for the first time distinct heterospory, structural as well as functional, is evident in pteridophytes, but of the several hundred forms in the group, only 9 genera (*Selaginella*, *Isoetes*, *Stylites*, *Marsilea*, *Pilularia*, *Regnellidium*, *Salvinia*, *Azolla*, and *Platyzoma*) are heterosporous.

In the Devonian fossil flora, heterospory has been found frequently in Lycopodiophytes also.

In heterosporous genera, there is the formation of two types of haploid spores within two types of sporangia: large, fewer-numbered *megaspores*, which develop via meiosis in the *mega sporangium*, and small, more numerous *microspores*, the products of meiosis in the *microsporangium*.

Each megaspore develops into a female gametophyte that bears only archegonia; a microspore develops into a male gametophyte, bearing only antheridia.

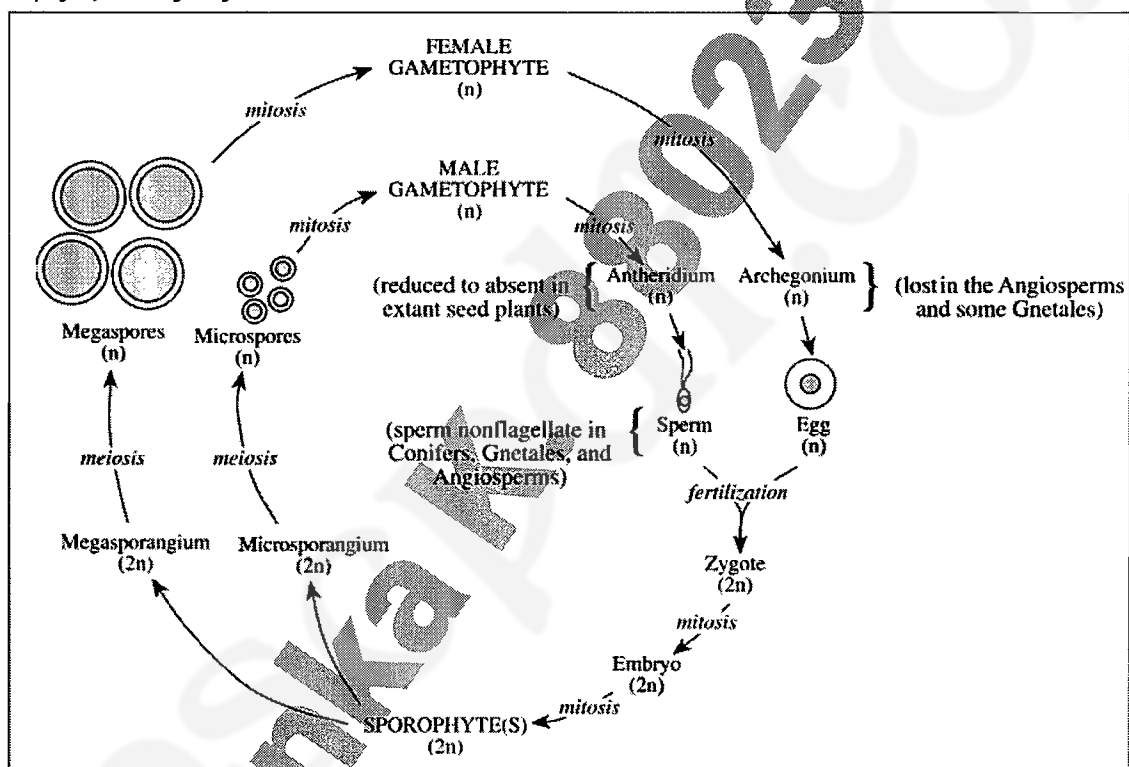


Figure 1: Life cycle of a heterosporous plant

Heterospory results from expression of one sex and repression of other in particular regions. Plants with sex chromosomes have a direct cytological basis for sex expression but none of heterosporous pteridophytes are reported to have sex chromosomes (Lewis & John, 1968).

Incipient Heterosporous Forms: Some Pteridophytes show incipient heterospory that is despite being homosporous they display tendencies towards heterospory. Important examples are discussed below.

1. *Equisetum* is a homosporous form but produces two types of gametophytes; smaller ones are male and larger ones female. The latter become hermaphrodite if fertilization is delayed. In a population the proportion of male and female gametophytes is influenced by environmental conditions. Sex determination, therefore, seems to occur during vegetative phase of gametophyte.
2. *Ceratopteris thalictroides*, a monotypic water fern, is another homosporous form that produces male and female gametophytes. However, the proportion of male and female gametophytes is not influenced by environmental conditions and female gametophytes produce antheridia if fertilization is delayed.

Uniqueness of Heterospory in *Platyzoma*: *Platyzoma*, a Queensland fern, is intermediate between forms showing incipient heterospory and complete heterospory. Sporangia are similar in size and occur

intermingled but spore size and number of spores in each sporangium is different. There are sixteen megaspores in a mega sporangium; these are twice the size of 32 microspores in a microsporangium. Microspores germinate to produce filamentous male gametophytes and megaspore form archegoniate gametophytes that develop antheridia if fertilization is delayed.

Developmental basis of heterosporous

In the absence of sex determination process, in heterosporous pteridophytes (Lewis & John, 1968), the developmental events explain the phenomenon of heterosporous. The differences between microspores and megaspores are not there not only in terms of size and sex-expression but also in terms of food reserves, organelles, nuclear shape and wall construction (Sussex, 1966).

A study of the forms showing heterosporous indicates that the most significant events in the determination process are:

- Time of sex determination.
- Number of archesporial cells in a sporangium; whether the archesporial cells directly differentiate as sporocytes or divide mitotically thereby increasing the number of sporocytes.
- Number of sporocytes initiating and completing meiosis.
- Number of spores attaining maturity.

In *Selaginella*, the micro- and mega sporangia are similar until the sporocyte stage. All the sporocytes in a microsporangium undergo meiosis and produce large number of microspores. In a mega sporangium most of the sporocytes degenerate leaving only a few to undergo meiosis and in most of the species only one sporocyte survives. After meiosis further difference may occur in spores. In most species four functional megaspores result, but in some the four spores are of different dimensions; the tetrad may result into two large and two small spores (*S. stenophylla*); on one large and three small spores (*S. molliceps*) or as an extreme a single large 1 functional megaspore with no trace of degenerates (*S. erythrospora*). In a collection of *Selaginella* sp. the botanists have observed presence of larger spores in microsporangia.

In *Isoetes* also, microsporangia and mega sporangia are similar and micro- and megasporophylls are indistinguishable except that they occupy different positions on the plant. In Microsporangia most of the sporogenous tissue differentiates as sporocytes and all the sporocytes complete meiosis to form microspores. In mega sporangia only a small number of deep seated archesporial cells differentiate as sporocytes and form megaspore tetrads.

Introduction to seed habit and its advantages

Evolution of seed structure was one of the most essential requirements of the terrestrial habit and the most advantageous adaptations in Phanerogams to combat terrestrial habitat.

Developmentally a seed is a fertilized ovule, which consists of a miniature, undeveloped plant (the embryo), which, alone or in the company of stored food [for its early development after germination] is surrounded by a protective coat.

The superiority of dispersal by means of seeds over the more primitive method involving single-celled spores lies in several factors.

- It gives full protection to the embryo due to the protective coat.
- The seed stores a good amount of food material thus offering parental care to the embryo. The stored reserve of nutrient material that gives the new generation an excellent growing start.
- Seeds are frequently small in size. As a result, they can be produced in large numbers and contribute profoundly to multiplication—almost with a comparable efficiency of free spore dispersing plants.
- Small size of seeds is also an advantage for wider dispersal as it can be carried across long distances.
- The seeds make negligible demands upon their environment and can survive under a wide variety of conditions.
- As most seeds also display dormancy, which is a state of arrested development, they can perennate efficiently under a wide variety of conditions – usually for periods much longer than what free spore can do.
- Due to the protective coat and a state of arrested development, the seeds can be dispersed across long distances without any damage to the embryo.
- The seed's multi-cellular structure provides ample opportunity for the development of adaptations for dispersal, such as plumes for wind dispersal, barbs, and others.
- Allowing for a dormancy period, the seeds enable surviving seasons of stress such as winter or low water. The germination of the seed occurs only under favorable conditions, enhancing survival of the new plant.

Undoubtedly, the seeds equipped the spermatophytes with the best and most efficient means of propagation. As a matter of fact, pollination and the "seed habit" are considered the most important factors responsible for the overwhelming evolutionary success of the flowering plants, which number more than 300,000 species.

Origin of seed habit

The earliest seed plants were the Gymnosperms, which arose late in the Devonian about 365 MYA.

The Gymnosperms evolved in the Late Devonian with a change in reproductive habit, the shift from a heterosporous life cycle to that of the seed. The oldest reported seed fossils, *Archaeosperma arnoldii* and *Elkinsia polymorpha* are about 350 MY old. They have been found in association with Pteridospermous elements (foliage, permineralized axes) such as *Triphyllopteris* and *Calamopitys*.

The evolution of the seed involved several steps. The exact sequence of these is not certain, and two or more steps in seed evolution may have occurred concomitantly.

However, the botanists today almost unanimously agree that heterospory was the first step in the evolution of seeds.

The steps involved in seed evolution have been as follows.

1. **Heterospory.** Heterospory is the formation of two types of haploid spores within two types of sporangia: large, fewer-numbered megaspores, which develop via meiosis in the mega sporangium, and small, more numerous microspores, the products of meiosis in the microsporangium. Each megaspore develops into a female gametophyte that bears only archegonia; a microspore develops into a male gametophyte, bearing only antheridia.
2. **Endospory.** Endospory is the complete development of the female gametophyte within the original spore wall. The ancestral condition, in which the spore germinates and grows as an external gametophyte, is called exospory.
3. **Reduction of megaspore number to one.** Reduction of megaspore number occurred in two ways. First, there evolved a reduction in the number of cells within the mega sporangium that undergo meiosis (each termed a megasporocyte or megaspore mother cell) was reduced to one. After meiosis, the single diploid megasporocyte gives rise to four haploid megaspores. Second, of the four haploid megaspores produced by meiosis, three consistently abort, leaving only one functional megaspore. This single megaspore also undergoes a great increase in size, due to the increased availability of space and resources in the mega sporangium.
4. **Retention of the megaspore.** Instead of the megaspore being released from the sporangium, in seed plants it is retained within the mega sporangium. This was accompanied by a reduction in thickness of the megaspore wall.
5. **Evolution of the integument.** The final event in seed evolution was the envelopment of the mega sporangium by tissue, called the integument. The integument grows from the base of the mega sporangium and surrounds it, except at the distal end where a small opening called micropyle is present. Fossil evidence suggests that integuments may have evolved first as separate lobes. In all extant seed plants, however, the integument develops as a continuous sheath. The micropyle functions as the site of entry of pollen grains or pollen tubes, which cause fertilization of the egg.
6. **Pollen grains.** Simultaneous to the evolution of the seed was the evolution of pollen grains. A pollen grain is, technically, an immature, endosporic male gametophyte. Endospory in pollen grain evolution was similar to the same process in seed evolution, involving the development of the male gametophyte within the original spore wall. Pollen grains of seed plants are extremely reduced male gametophytes, consisting of only a few cells. They are termed immature male gametophytes because, at the time of their release, they have not fully differentiated. After being released from the microsporangium, pollen is transported to the micropyle of the ovule (or, in angiosperms, to the stigmatic tissue of the carpel) in order to ultimately cause fertilization.

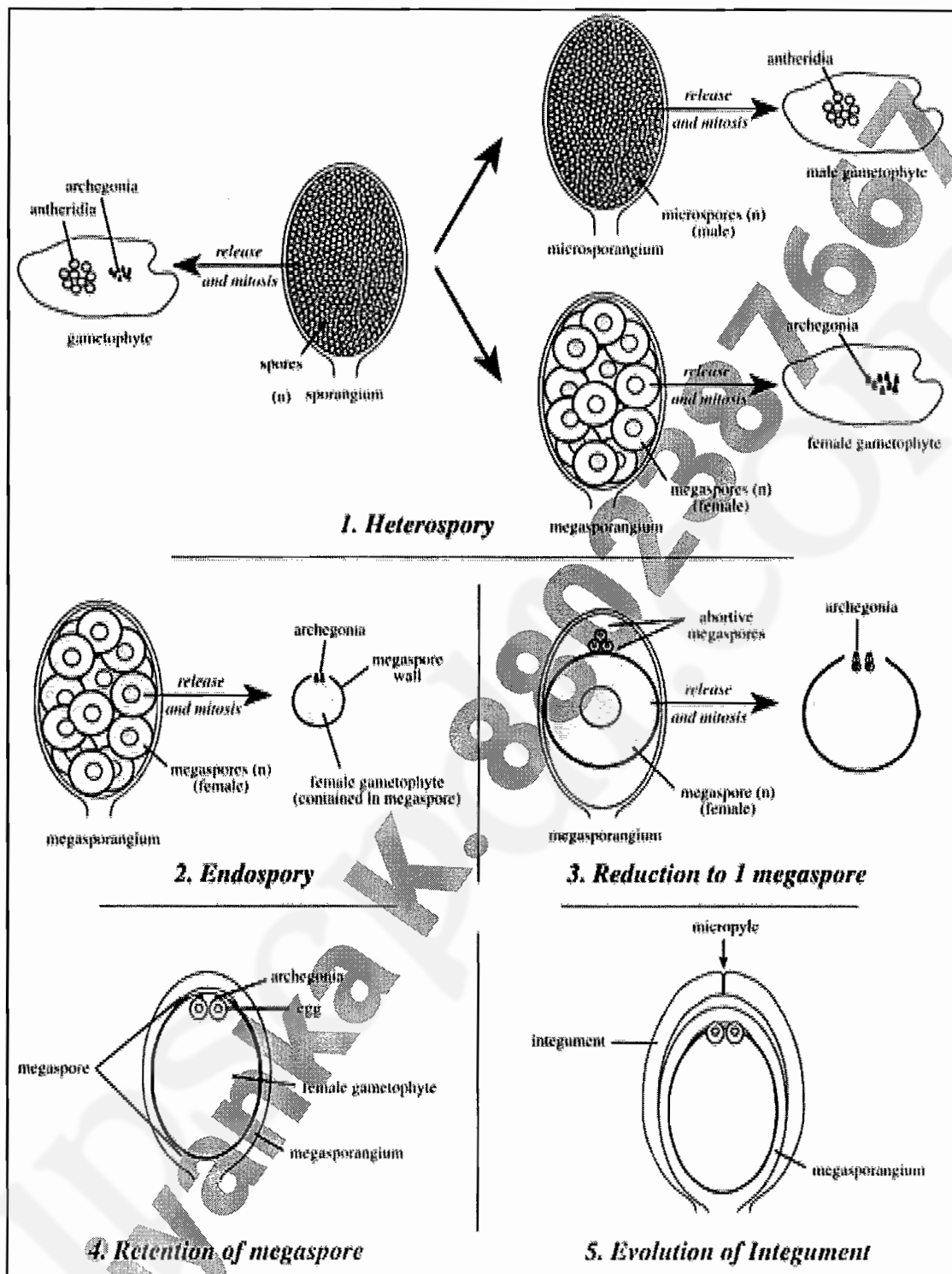


Figure 2: Transition from heterospory to seed habit

Heterosporous Pteridophytes showing transitional features

Pteridophytes are essentially non-seeded plants. Most of them are homosporous. However, as discussed earlier, a few pteridophytes are heterosporous e.g. *Selaginella*, *Isoetes*, *Marsilea*, *Azolla*, *Salvinia*, *Pilularia*, *Regnellidium*.

Some of these heterosporous pteridophytes possess some of the characteristics needed for seed formation. For example, a species of *Selaginella* possesses the first four of the above mentioned requirements. As a

matter of fact, in most of the species of *Selaginella*, the megaspore is retained within the mega sporangium. It germinates *in situ* and grows upto an advanced stage of female gametophyte. In *S. kraussiana*, the first archegonial initial is set up before the semi-germinated megaspore is shed. Further advancement has been achieved by *S. rupestris*, where only one megaspore is produced within the mega sporangium. It grows and produces a well developed female gametophyte, which after fertilization forms an embryo and the megaspore is still enclosed within the mega sporangium and attached to strobilus. The embryo develops shoot apex, cotyledons and roots. This stage can be compared with the phenomenon of vivipary that occurs in some angiosperms.

However, the final requirements of seed habit development are missing in all species and hence *Selaginella* fails to produce seeds.

Despite this, there are several evidences which establish that the heterosporous pteridophytes have been ancestors to the seed plants.

1. There is a considerable similarity between *Selaginella* female gametophyte with the embryo is within mega sporangium and the longitudinal section of the ovule of a primitive gymnosperm e.g. *Lagostoma*.
2. The mega sporangium of *Stauropteris burntislandica* presents several exceptional features, pertinent few of them are as follows:
 - a. Basal half or more of the sporangium is sterile possibly serving as food reservoir,
 - b. A slender vascular strand running through the sterile portion. This is the most extensive vascularization of any fern or fern like sporangium,
 - c. Few specimens show a tapering apex with a minute opening.

It is a specialized mega sporangium but not a seed. Accordingly a seed can be conceived (Andrew, 1966) to have evolved from it, involving the three steps.

- a. Reduction in number of megaspores to one and increase in size of the survivor,
- b. Sinking of megaspore towards the basal part of mega sporangium.
- c. Displacement of the vascular strand and its division into two branches which developed around the megaspore.

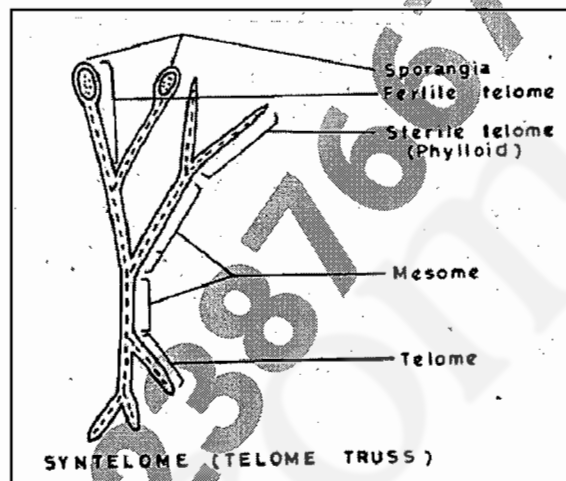
Telome Thoery

Telome Theory

Telome theory was first proposed by Walter Zimmermann in early 1930s and later elaborated by him in 1938, 1952, 1959 and 1965.

According to this theory, all vascular plants have evolved from a simple, leaf-less, root-less and dichotomously branched sterile and fertile axes called telomes. Such an ancestral plant could be *Rhynia* or its close relative. The other plants developed leaves, roots, sporangia and other organs by modification and differentiation in telomes.

Zimmermann termed the extreme terminal portions of plants, having single nerves, as telomes and the internodes below it as mesomes. The telome may be fertile, having single terminal sporangium, or sterile without sporangia. A plant axis is thus composed of telomes and mesomes. A group of telomes is known as telome truss.



The Telome Modification Processes

According to the telome theory, various organs viz. leaves, roots, sporangia, etc., have evolved from simplest and earliest vascular plants by progressive differentiations, called 'elementary processes'. These are briefly discussed below:

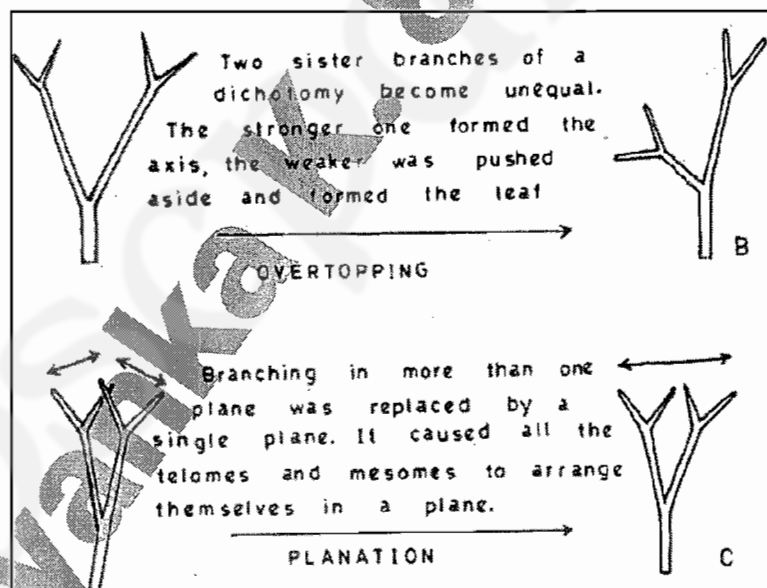


Figure 1: Telome Modification Process

Overtopping: During this process the two sister branches of a dichotomy (or telome truss) became unequal. One of them became stronger and grew erect, while the other became weaker and pushed aside. The stronger branch became axis and the lateral branch became lateral appendage or leaf. This process was repeated several times and resulted sympodial axis which later became monopodial.

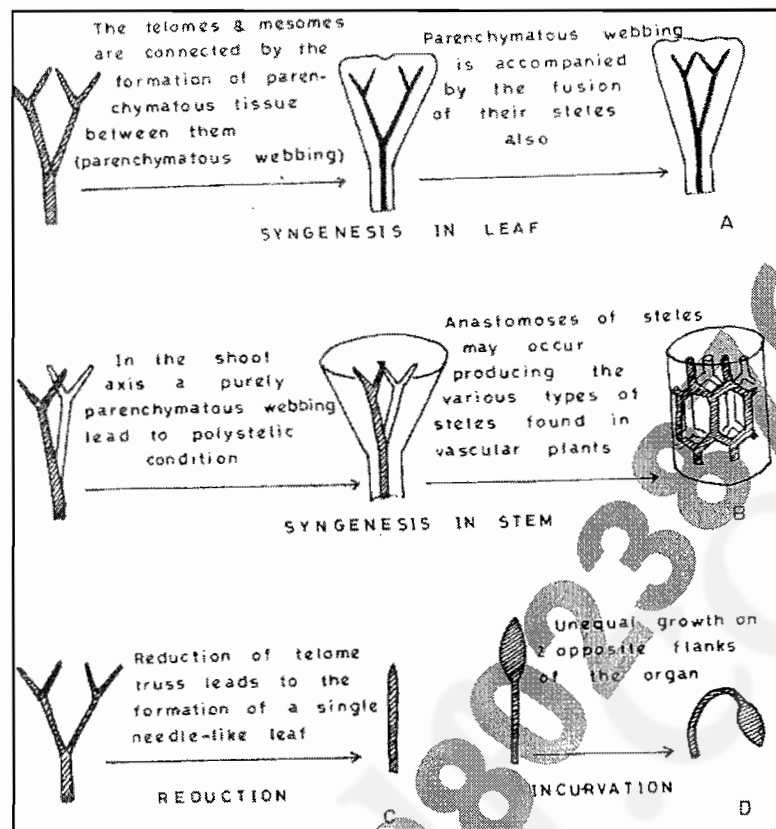


Figure 2: Telome Modification Processes - II

Planation: During this process the axis divided dichotomously in more than one planes. These branches were arranged in single plane and gave rise to leaves.

Syngensis (or webbing): During this process the telomes and mesomes became fused by the formation of parenchymatous webbing in between them. This resulted in the formation of—(a) leaves with open dichotomous venation, (b) leaves with pinnate venation, (c) leaves with reticulate venation and (d) shoot axes with various types of steles viz., actinosteles, siphonosteles, solenosteles and eusteles.

Reduction: Simplification and reduction of telome-truss resulted in the formation of microphylls and needle-shaped leaves. Reduction would result if the activity of terminal meristem of telome truss was suppressed so that the length of telome would become short.

Incurvation or Recurvation: During this process the fertile telomes were reflexed so that the sporangia became inverted in position, e.g., inward-projecting sporangia on a sporangiophore of *Equisetum*.

Examples of telome modifications

Origin of leaves

According to Zimmermann's Telome theory, the microphyllous leaves as in lycophyta originated by the process of reduction and the megaphyllous leaves (as in filicophyta) originated by combined processes of overtopping, planation and syngensis.

Origin of sporophylls in Lycophyta

The sporangia, in the members of lycophyta, are usually borne in the axils of microphyllous sporophylls. It resulted by aggregation of fertile and sterile telomes, followed by reduction in the mesomes and number of sporangia. It resulted in the formation of single sporangium in the axil of single microphyllous sporophyll. An intermediate stage can be seen in the extinct genus *Protolopododendron*.

Origin of sporangiophores in Sphenophyta or Arthropophyta

The members of this division are characterised by having sporangia borne on peltate sporangiophores. According to this theory, the sporangiophores originated by the combined processes of— (a) overtopping and reduction in the fertile segments resulting whorled arrangement, (b) recurvation of fertile branches

leading to the downward bending of sporangia, and (c) syngensis and expansion of telomes and mesomes leading to the formation of pletate sporangiophores.

Merits of Telome Theory

1. The theory is simple and explains all morphological differentiations in living as well as extinct plants of various groups.
2. It clearly explains origin and evolution of sporophytes of vascular plants.
3. This theory is based mainly on comparative study of fossil as well as living genera of vascular plants.
4. Most of the assumptions of this theory are based on exact phylogenetic relationships between the various groups of plants. It is also supported by recent molecular phylogenetic studies on land plants (Kenrick & Crane, 2000; Linda Graham *et al* 2002).
5. The elementary processes (i.e., overtopping, plantation, syngensis, reduction and incurvation) clearly explain the manner of evolution in the direction of simple as well as complex vascular plants of present day.

Demerits of Telome Theory

Telome theory of Zimmermann has been well accepted by most of the botanists, but criticized by Bower (1946), Thomas (1950), Takhtazan (1953), Andrews (1960), Foster (1950), Puri (1947, 1951) and others. The main points of disagreement are following —

1. Recent molecular phylogenetic studies on land plants clearly establish that the Lycopside represent a distinct evolutionary line. They are not derived from the *Rhynia* stock.
2. According to Bower (1946), the theory took for granted the telome as a fundamental unit without clearly mentioning as to how such a unit has acquired its characteristic development.
3. Thomas (1950) observed the presence of whorled sporangia in some primitive land plants. Presence of whorled sporangia has not been mentioned in the telome.
4. According to Eames (1936), the origin of leaves in lycopside can be regarded as simple outgrowths of axis and does not agree the way it has been explained in the telome theory.
5. Andrews (1960) disagrees with the telome theory as for the evolution of some of the ferns is concerned.

Rhyniophyta

What are the Rhyniophytes?

The Rhyniophyta is the group of earliest vascular plants. They are called so because they were originally described from the Rhynie Chert in Aberdeen shire, Scotland. The group originated in the mid-Silurian. Their earliest fossil record corresponds to *Cooksonia* of Silurian age, a 428 million year old fossil. It is widely accepted now that the Rhyniophytes originated from *Chara* like ancestors.

The group included the members of the monophyletic Rhyniopsida, *Cooksonia* and *Rhynia* as well as on two well known related genera, *Horneophyton* and *Aglaophyton*. Thus, the Rhyniophytes are a paraphyletic taxon comprising the first vascular land plants (Rhyniopsida) and their morphologically similar relatives.

They are considered to the basal group in the evolution of several vascular plant groups, namely the Trimerophyta, Ferns and Progymnosperms. Thus, they have a key position in the plant evolution.

Features of the Rhyniophytes

1. Presence of vasculature, mostly haplostelic type in the Rhyniopsida members but presence of moss like conducting cells in the genera related to Rhyniopsida (viz. *Aglaophyton* and *Horneophyton*). Thus strictly speaking, the genera fall not exactly within Rhyniopsida but they are related to non-vascular ancestors and display protrachaeophyte stage, i.e. structural features transitional between aquatic non-vascular and land vascular plants (Kerp, 2006).
2. No seed formation
3. Simple plant body, with a dichotomously branched cylindrical stem, rhizoids and no leaf.
4. Rhizoids as the underground structure for anchorage and absorption
5. No leaves but some hemispherical protuberances on the upright axis
6. The plant axis branched dichotomously and it could be partially horizontally spreading and partially vertical.
7. The vertically growing aerial axis had cuticle covering and stomata
8. Dispersal by means of spores, produced homosporously but, it has been recently established that *Aglaophyton* was heterosporous (Taylor *et al*, 2007).
9. Terminal sporangia, developing on branch tips
10. Most members marsh inhabitants

Genus *Rhynia*

Rhynia is a now extinct genus with great evolutionary significance, belonging to the class Rhyniopsida of Rhyniophyta. *Rhynia* was one of the first and the most abundant Rhynie chert (in Aberdeenshire, Scotland) plants to be described.

Explanatory Note

1. The Rhynie Chert is one of the most important fossil plant occurrences because it represents the oldest and most completely preserved terrestrial ecosystem. The Rhynie Chert has been radiometrically dated at 396 Ma (Pragian). Plants remains show excellent cellular preservation. Three groups of plants are represented: the Rhyniophytes, the Zosterophyllophytes and a lycopod.

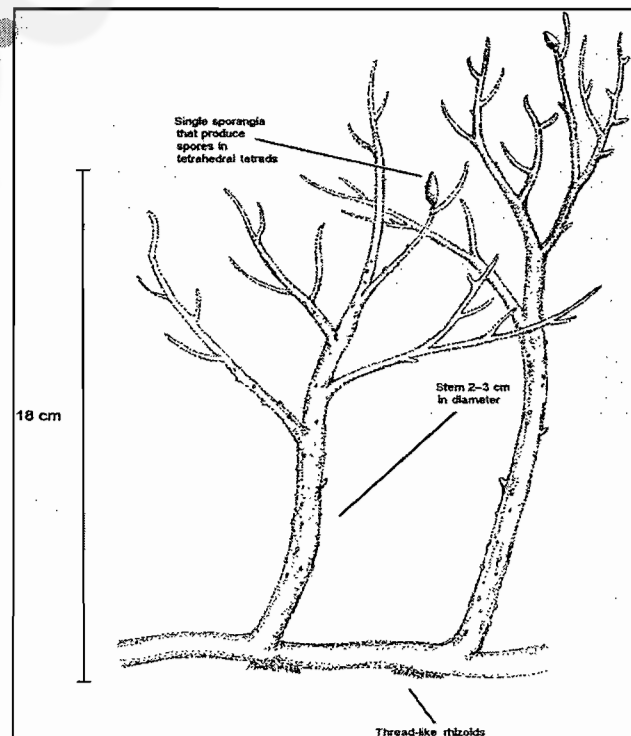


Figure 1: *Rhynia Gwynne-vaughanii*

The plant was originally described and classified by Kidston and Lang in 1917, 1920 who assigned the species name *Rhynia gwynne-vaughanii*.

Kidston and Lang also identified another species called *Rhynia major*; but this plant is now assigned to a separate genus *Aglaophyton*, because of a very different vascular anatomy (which shows the protetracheophyte stage).

Rhynia gwynne-vaughanii

Sporophyte Structure

The sporophytic plant body of *Rhynia* is divided into three parts:

1. **Branched aerial axis** that does not bear any leaf, but has **sporangia at its tips**.
2. **Horizontal Rhizome**
3. **Rhizoids** providing basic rooting system

1. **The aerial axis** of *Rhynia* exhibit a maximum diameter of 3 mm and the plant probably attained a height of up to 20 cm. The aerial or rather the 'upright' axes are cylindrical, naked and upwardly tapering. The branching of *Rhynia* is both **dichotomous** and **adventitious** or **monopodial**, with dichotomy occurring at an angle between 17 and 35° (D. S. Edwards 1980). There was **no leaf**.

The surface of the axis bear numerous conspicuous emergences or **hemispherical projections** from the epidermis which are occasionally located beneath stomata and at the base of adventitious branches and in other instances internally display fungal activity and dark necrotic tissue.

The **stomata** typically appear circular on the cuticle surface and are flanked by two guard cells. The cells of the **cuticle** often exhibit a median ridge giving the cuticle a flanged appearance.

The **sporangia** of *Rhynia* are not particularly common. They are fusiform, displaying a maximum size of 3.6 mm by 2.4 mm. The disposition of the sporangia is terminal, being located on the adventitious branches of fertile aerial axes. No dehiscence mechanism has been observed though a dark cellular layer or 'sterile pad' at the base of the sporangium has been interpreted as a site of abscission by D. S. Edwards (1980). The sporangial wall comprises three layers: an outer epidermis, a poorly preserved parenchymatous layer and an inner tapetal layer. The vascular tissue or stele is **protostelic** and comprises a zone of 'phloem' of uniform thickness surrounding a central xylem strand.

2. **The Rhizomal Axis:** *Rhynia* laid directly on the ground surface. *Rhynia* possesses a creeping rhizome displaying repeated dichotomous and adventitious branching, locally turning upright, passing upwards into the 'aerial' axes. The rhizomal axes are cylindrical and naked and generally exhibit a similar morphology and internal anatomy to the aerial axes though they lack stomata. The other main difference is exhibited by the hemispherical projections, which commonly support tufts of **unicellular rhizoids**.

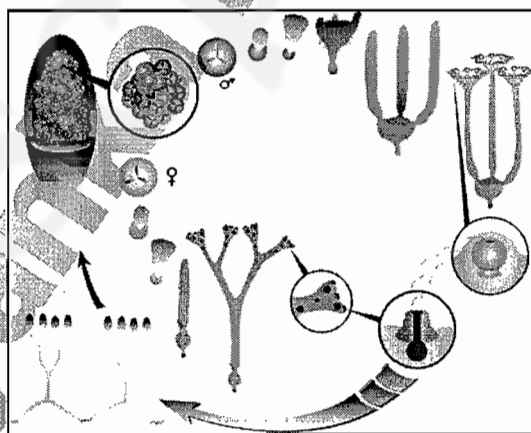


Figure 2: Life history reconstruction of *Aglaophyton* (after Thomas N. Taylor, Hans Kerp and Hagen Hass, 2005) showing stages in the development of the dimorphic gametophytes. The mature sporophyte bears sporangia with spores of two types.

Gametophyte Structure

Information about the gametophytic phase in *Rhynia* remains speculative as no fossil remain have been found for the same. Unequivocal gametophytes of *Aglaophyton* have recently been discovered in 2005 (Thomas N. Taylor, Hans Kerp and Hagen Hass, 2005).

Gametophytes of *Aglaophyton* were initially described as *Remyophyton delicatum*. The gametophyte development for *Remyophyton delicatum* is described in four stages: (i) globular, (ii) teardrop-shaped, (iii) protocorm, and (iv) gametangiophore; as shown in Figure 2.

Evolutionary importance of *Rhynia*

The Telome Theory of Walter Zimmermann given in 1933, has proposed that the complex land plant forms have arisen from *Rhynia* and its relatives. From the simple morphology of these Silurian–Devonian fossil members, it is hypothesized that early land plants possessed a common set of developmental processes centered on primary growth of shoot apical meristems.

For more than 50 years, the telome theory has been extremely influential in interpreting the evolutionary history of land plant architecture.

It is indeed believed today that the more complex land plants (at least two thirds of them) have arisen from *Rhynia* like ancestors; although their process of development must have been more complex than what is explained by Telome Theory.

The evolutionary biologists also agree that there must have been other ancestral groups involved in giving rise to the vascular land plant diversity that we see today.

The Psilotales

The Psilotales are the least complex of all terrestrial vascular plants, and were once believed to be remnants of an otherwise extinct Devonian flora. This is primarily because psilophytes are the only living vascular plants to lack both roots and leaves. Though they have been considered "primitive," recent developmental and molecular evidence suggests that the group may actually be reduced from fern-like ancestors. There is a growing universal agreement on this [Hass, 1996].

Despite the uncertainty of their relationships, psilophytes do structurally resemble certain early vascular plants, and are used as a model for understanding the ecology of these plants.

This is a small group with only two genera, *Psilotum*, and *Tmesipteris*, neither with many species. Both genera grow in tropical or subtropical regions, where they occur on rich soil or as epiphytes. *Psilotum* occurs in North America in the Caribbean, and along the Gulf and Atlantic Coasts to as far north as North Carolina, and has been reported from one locality in Arizona. It may also be found in tropical Asia and on Pacific islands. *Tmesipteris* grows in New Caledonia and nearby areas of the South Pacific, including Australia and New Zealand. In addition to its natural distribution, *Psilotum* is also found as a common weed in greenhouses, and sometimes escapes cultivation in regions with mild climate. It occasionally becomes a nuisance, but is still very popular for its unusual growth form. In Japan, more than 100 unusual breeds have been produced, some of them highly prized by cultivators.

The Psilotales have no fossil record at all.

Morphology and Life History of Psilotales

The psilophyte stem lacks roots; it is anchored instead by a horizontally creeping stem called a **rhizome**. The erect portion of the stem bears paired **enations**, outgrowths which look like miniature leaves, but unlike true leaves, the enations have no vascular tissue. These paired outgrowths lie immediately below the spore-producing **synangia**, which produce the spores. The synangia appear to be the product of three sporangia which became fused over the course of evolution, and are borne on the tip of a short lateral branch. This is another feature in which the psilophytes differ from other living vascular plants; all other such plants produce their sporangia on their leaves.

When the synangia mature, they open to release yellow to whitish spores, from which the **gametophyte** plants will later emerge, like the one shown at left. The gametophytes are very small, usually less than two millimeters long. They are subterranean and **saprophytic**, getting their nutrition by absorbing substances dissolved in the environment. This is often aided by the presence of fungi which grow into the tissues of the gametophyte and through the surrounding soil.

Eventually, the gametophyte reaches sexual maturity, producing both egg and sperm cells. The multiflagellate sperm swim to the egg cells, where they unite to begin the **sporophyte** generation. Psilophyte gametophytes may even self-fertilize to produce a sporophyte plant. The resulting sporophyte begins its life as a dependent on its parent gametophyte, as in other seedless plants. But unlike the "bryophytes," the sporophyte eventually gains independence from its parent, and establishes itself in the environment.

The mature sporophyte of *Psilotum* will often grow to 30 cm tall, and may grow even taller. It has no true leaves, and instead the stem is green and photosynthetic, being covered with **stomates** to allow gas exchange. As the cross-section at right shows, the stem has a central core of vascular tissue (**protostele**) which is usually lobed. The thick-walled cells in the center of this core are sometimes considered to be pith, in which case the vascular arrangement would actually be a **siphonostele**. Surrounding the vascular tissue is a layer called the **endodermis**, which has specially packed cells to regulate flow of water and nutrients.

Tmesipteris has similar reproductive structures and life history to that of *Psilotum*, but by contrast it has broad leaf-like extensions of its stem, each with a single vascular bundle. These extensions may lie to either side of the stem, forming a flat growth, or they may be radially arranged. In any case, they are not considered leaves by most botanists, though this interpretation has been challenged by some workers.

Psilotum

Rhizomatous, spore-bearing vascular plant 2-3 feet tall, native to southeastern United States, Japan and Australia.

This genus is one of only two genera in the whole group Psilotales.

Psilotum is also called whisk fern. Whisk Fern has very primitive morphology. It best represents what some of the first vascular land plants in the Devonian would have looked like. It has only dichotomously

branching stems, sporangia, and leaf-like enations. In that respect it resembles the other Devonian vascular plants as *Rhynia* and *Cooksonia*.

Characteristic features:

- The sporophyte plant body is differentiated into a subterranean prostrate branched rhizome and dichotomously branched erect aerial axis.
- Sporophyte lacks root, water absorption is performed by large rhizoides borne on rhizome. The rhizomes commonly have mycorrhizal association.
- The aerial shoots lack true leaves and may bear scale – like or small leaf- like appendages.
- Vascular cylinder consist of central solid core of xylem surrounded by phloem consist of thin walled vertically elongated living cells.
- Pith and cambium are totally absent. There is no secondary growth

In the reproductive cycle of whisk ferns, a dominant sporophyte plant (1), bearing trilobed sporangia (2), produces homosporous spores (3) which germinate into haploid gametophytes (4). The underground gametophyte forms mycorrhizal associations with fungi for nutrition and develops archegonia (5), each with 1 egg, and antheridia (6) with flagellated sperm. After fertilization a diploid zygote (7) forms and eventually develops into a sporophyte plant (8). Asexual reproduction can take place by means of gemmae. While gemmae are means of asexual reproduction in non-vascular plants such as the liverworts, the difference is that in the non-vascular plants the gemmae develop on the gametophyte while in *Psilotum* the gemmae develop on the sporophyte.

Recent molecular evidence suggests, however, that *Psilotum* is not as primitive as most people believed and is related to ferns.

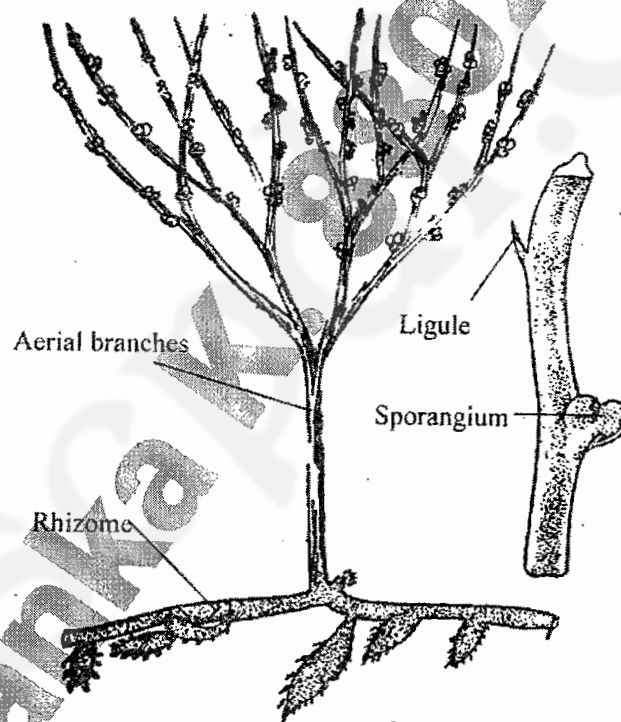


Figure 1: *Psilotum* sporophyte

Lycopodium

LYCOPODIUM

The genus *Lycopodium* includes 287 described species (Watson & Love, 2001) which are distributed throughout the world mostly common in tropical and sub-tropical forests. So far, 32 species have been described from India. 11 of them are endemic. Most of the species are terrestrial, which grow on humus rich soil in moist and shady places (e.g. *L. cernuum* *L. selago* *L. serratum* etc.) A few species are epiphytic and grow on tree trunks (e.g. *L. phlegmaria* *L. squarrosum*, etc.). Some sp. grow as climbers (e.g. *L. volubile*)

The species of *Lycopodium* show a wide range in the growth and form of saprophytes. This led taxonomist to divide the genus into a number of sub-genera. On the basis of general organization of saprophyte Pritzel (1900) divided the genus into two subgenera – (i) *Urostachya*. Which includes common species like *L. Selago* *L. phlegmaria* *L. Squarrosum* *L. pithyoides* etc. and (ii) *Rhopatostachya*. Which includes common species like *L. clavatum* *L. cernuum* *L. inundatum* *L. volubile* etc.

Structural Aspects

The adult saprophytic plants are delicate. Evergreen perennial herbs or shrubs with a wide range in habit and habitats. Most of the species are terrestrial, while a few are epiphyte on tree trunks. The terrestrial species may have erect axes or prostrate creeping rhizomes. Which gives rise to erect aerial branches? The plants are differentiated into root.

Stem and leaves

Stem: The stem of *Lycopodium* is delicate weak slender and cylindrical. The branching is dichotomous. The dichotomy is usually unequal, sometimes called as pseudomonopodial in some species.

Root: The roots are usually adventitious; arise endogenously from the base of an erect axis or from the entire length of prostrate axis. They are dichotomously branched and arise in acropetal succession. In some species the roots initiate endogenously near the shoot apex, grow downwards in the cortex and finally emerge out near the level of soil (e.g. *L. selago* *L. pithyoides*, etc.)

Leaves: The leaves are simple, sessile with broad bases, small (i.e. microphyllous), reaching up to 2-10 mm in length (but may attain the length up to 3 cm in some cases). Each leaf is provided with single up branched vein. They are usually arranged spirally on the stem but in some cases they may be whorled (e.g., *L. cernuum*) or opposite decussate (e.g. *L. alpinum*)

The leaves are usually alike (i.e. homophyllous) but a few species especially the epiphytic (e.g. *L. complanatum*) show heterophylly. The leaves of lateral rows are whereas those of upper and lower rows are smaller. The fertile leaves are called sporophylls.

Internal features of the stem

A transverse section of stem shows the following internal structures –

Epidermis: The epidermis is single layered, interrupted with stomata. The outer walls of epidermal cells are cutinized.

Cortex: It varies in thickness from species to species. The cortex, in most of the epiphytic pendent and erect species is homogenous consisting of only thin walled parenchymatous cells (e.g. *L. phlegmaria* *L. selago* etc.). In some cases, the mature stem has sclerenchymatous cells in whole of the cortex. In others, the cortex is differentiated into three zones-outer, middle and inner zones. In *L. clavatum*, the outer and inner cortexes are sclerenchymatous whereas middle cortex is parenchymatous. In case of *L. cernuum*, the outer and inner cortexes are parenchymatous whereas middle cortex is sclerenchymatous. The cortex may show presence of leaf traces. Each leaf trace consists of 3-8 spiral and annual xylem tracheids surrounded by a ring of thin-walled cells.

Endodermis: The endodermis is single layered, the cells of which have a distinct casparian strip. It is not well differentiated in mature stems.

Pericycle: The endodermis is followed by one or many layered pericycle consisting of thin w-walled compact parenchymatous cells.

The stele: The stele in all the species is a protostele (i.e., the vascular elements from a solid axial cylinder in which the central core of xylem is surrounded by phloem). It varies with respect to the shape and the arrangement of avuncular elements (xylem and phloem) in different species. Various kinds of protosteles found in different species of *Lycopodium* are listed below:

- (i) **Actinostele:** The xylem core is more or less stellate (star shaped) in section, with phloem lying between the arms. The metaxylem lies towards centre whereas the protoxylems are situated at the tips of star shaped projections towards periphery. Thus, the xylem is exarch. Such type of stele is found in the spore lings of all the species of *Lycopodium* and mature stems of *L. selago*. *L. phlegmria*. *L. serratum*. etc.
- (ii) **Plectostele:** In a transverse section, the xylem and phloem appear in the form of parallel bands alternating with each other. These bands appear inter-connected in a longitudinal section. Such state is found in mature stems of *L. Clavatum* *L. volubile*. etc.
- (iii) **Mixed protostele:** The xylem and phloem tissues are uniformly distributed, in the mixed protostele. In a transverse section, the xylem patches appear to be embedded in a mass of phloem. Such stele is found in mature stems of *L. cemuum*.

In all cases differentiation of xylem begins at the exterior and proceeds towards centre. Thus, the protoxylem is uniformly exarch. The protoxylem consists of tracheids having annular or spiral thickenings. The metaxylem tracheids may show scaalanform thickenings of circular bordered pits. The phloem consists of sleeve cells and phloem parenchyma. The sleeve plates occur on both the lateral and tapering end walls. All the vascular tissues are primary and there is no secondary growth or cambial activity.

Leaf

The internal structure of leaf is simple. It shows almost flattened (e.g. *L. selago*) or sometimes triangular outline (e.g. *L. clavatum*) in a transverse section.

The outermost single layered epidermis shows the presence of stomata on both the surfaces (i.e. amphistomatic), whereas a few species (e.g. *L. volubile*) show stomata only on the lower epidermis (i.e. hypostomatic). The epidermal cells are covered with cuticle. Internally the mesophyll is not clearly differentiated. It consists of rounded or oval chlorophyllose cells having numerous intercellular spaces. Each leaf is supplied with a single unbranched vascular strand. The vascular bundle has a central core of xylem tracheids surrounded by phloem. The protoxylem when distinct is situated centrally i.e. research.

Root

The root originate endogenously and branch dichotomously. They bear root caps and paired root hairs. A transverse section of root shows the following structure features-

Epidermis: The outermost single layered epidermis consists of thin walled cells. Some epidermal cells divide by oblique walls or anticlinal walls) and each one of them give rise a long unicellular root hair. Thus the root hairs arise in pairs.

Cortex: The epidermis is followed by a broad tone of cortex. It is differentiated into two regions outer and inner cortex. In most of the species (a. *L. selago*. *L. pithyoides* etc.) The outer cortex is thick-walled sclerenchymatous and inner cortex is thin-walled parenchymatous. In some cases (e.g., *L. clavatum*) the outer cortex is thin-walled paxenchymatous and inner cortex is thick-walled sclerenchymatous.

The cortex is followed by single layered endodermis, which is not well developed.

Stele: The stele is protostele. It is usually diarch or tetrarch or in some cases polyarch. In *L. pithyoides* the diarch stele shows crescent shaped xylem with protoxylem occupying it's both the ends ie. Exarch and the phloem occupying the position between both the ends of xylem. In *L. Selago*, it is diarch to tetrarch. In tetrarch condition. It shows two separate bands of exarch xylem with phloem occupying the position between them.

In some species (e.g. *L. clavatum*), the stele is very similar to that of the stem. It is polyarch, it consists of 6-10 radiating bands of xylem alternating with phloem. The protoxylem lies towards periphery and metaxylem towards centre. The metaxylem of these radiating plates unite in the centre. The stele is plectostele. Sometimes it books actinostelic protostele.

Reproduction

Vegetative reproduction

Vegetative reproduction takes place in different species of *Lycopodium* by the following methods —

- (i) **Reproduction by gemmae or bulbils :** Some special reproductive bodies called gemmae or bulbils are produced near the stem tips of sporophytic plant body, which serve as means of vegetative reproduction and found to occur in *L. selago*, *L. lucidulum* and *L. phlegmaria*, These are small, bud-like lateral outgrowths. Each gemma consists of a short axis surrounded by a number of thick wing-like leaves. It develops a root before falling on ground, which fixes the young plant to the substratum when the gemma detaches and falls.

- (ii) **Formation of resting buds:** In *L. inundatum*, the growing tips of rhizome store food materials and become resting buds. During unfavourable condition, the remaining portion of rhizome dies off except the resting buds. They resume growth and develop new plants at the onset of favourable conditions. Thus, the resting buds serve as the organs of perennation.
- (iii) **Root tubercles:** Reproduction by the formation of special multicellular tubercles arising from the cortical cells of roots have been reported in *L. cernuum* and *L. ramulosum*. These tubercles store food material and are protected by thick walls. They detach and germinate directly to give rise new plants.
- (iv) **Fragmentation :** Reproduction by the death and decay of the posterior older portion of stem leading to the separation of young branches occur most commonly in *Lycopodium*. These young branches separate and grow as new plants.

Reproduction by spores

THE SPORE PRODUCING ORGANS

The genus *Lycopodium* is **homosporous** (i.e., its spores are all alike in size, shape and germinative behaviour). It reproduces by the spores produced inside the sporangia. The sporangia are borne singly in the axil of special fertile leaves called sporophylls (e.g., *L. selago*, *L. inundatum*) or on the adaxial (upper) surface of sporophylls (e.g., *L. clavatum*, *L. cernuum*). In most of the species the sporophylls are aggregated into distinct strobili or cones, whereas in a few cases definite strobili are not formed. In *L. selago*, the strobili are not formed and the sporophylls (similar to vegetative leaves) are loosely arranged all along the erect branches. Usually there is a regular alternation of fertile zone and sterile zone on these branches (the condition called *Selago Condition*).

The strobilus or cone

An aggregation of sporophylls into a definite compact (or sometimes loose) body is called strobilus or cone. It is a modified upper portion of stem having short internodes bearing sporophylls. The shape and size of strobili vary in different species of *Lycopodium*.

In *L. clavatum*, the strobili are borne on special modified branches called **peduncles**. The sporophylls are small and closely appressed around strobilus axis. They possess small outgrowths or flange on abaxial side to protect the sporangia below.

In *L. cernuum*, the strobili are small, sessile and borne at the ends of leafy branches.

The strobili of *L. squarrosum* and *L. inundatum* are not distinctly marked off from the vegetative shoots because the sporophylls are almost similar to the foliage leaves.

THE SPORANGIA

Development of sporangium:

The development of sporangium, in all the species of *Lycopodium*, is of eusporangiate type i.e., it originates from a group of initial cells.

Structure of mature sporangium

Each sporangium is differentiated into two parts

1. the stalk and
2. the capsule

The stalk may be long and narrow (e.g., *L. selago*) or short and massive (e.g., *L. clavatum*). In *L. clavatum*, the broad and massive stalk extends into the sporogenous tissue in the form of *subarchesporial pad*.

The capsule is kidney-shaped, orange to yellow in colour and consists of only one locule (unilocular) enclosed by three or more wall layers. The wall of capsule is three or more layered. The innermost wall layer is called *tapetum*, which provides nourishment to the developing spores. The wall of fully mature sporangium consists of single layer of cells as the inner layers breakdown during maturation. The cavity of capsule is filled with a large number of homosporous spores.

Dehiscence of sporangium is by a narrow transverse strip of cells called **stomium** in the outermost wall layer. The cells of stomium show a very special type of cell-wall thickenings specially designed for the purpose of dehiscence. At maturity, when the strobilus loosens due to elongation of central axis and the sporangia come in contact with dry air, the outer layer loses water. As a result, the sporangium splits open along the line of dehiscence (i.e., stomium) in the form of two valves and the spores are scattered by wind.

THE GAMETOPHYTE

The sporophytic generation ends with the meiosis in spore mother cells and the gametophytic generation begins. The haploid spore is the first cell of gametophytic generation and germinates to produce gametophytic plant body (or *prothallus*).

The spore: The genus *Lycopodium* is homosporous (i.e. it produces only one type of spores). Each spore is small, approximately (0.03–0.05 mm) in diameter and distributed by wind. It has three triangular faces at its proximal pole called tri-radiate ridge (**trilete spores**). The spore wall is double layered—the outer etc may be smooth or with reticulate ornamentation and the inner granular intine. The spore wall encloses prominent haploid nucleus surrounded by cytoplasm laden with reserve food material in the form of fats and oils. The chloroplast may or may not be present.

Germination of spore and subsequent development leads to the formation of the prothallus.

Different types of prothalli in *Lycopodium*

In *Lycopodium*, the nature of spore wall determines the form and duration of gametophytic plant body (prothallus). Smooth walled spores germinate earlier and produce green and short-lived prothalli. On the other hand, rough spores take longer time (up to several years) to germinate and buried deep into the soil. They produce non-green, saprophytic, subterranean, longer-lived gametophytes. A great variation has been observed in the form and nature of prothalli in different species of *Lycopodium*. They have been categorized under three main types (Trueb, 1889; Bruchmann, 1916). These types are briefly discussed below —

First type

The prothallus is simple, upright (erect), cylindrical and fleshy. It is 2–3 mm in high 1–2 mm in diameter. It rows at the surface of ground with colourless lower portion embedded in the soil and green upper portion exposed to air. The upper portion is variously lobed, chlorophyllose and photosynthetic. Thus, the prothallus is independent because it can synthesize its own food (i.e. autophytic). The rhizoids arise from lower embedded portion, which also bears endophytic fungus (mycorrhiza). The prothallus grows by the activity of meristem, which is situated marginally below the apex. The sex-organs develop in the generative zone, situated below the green lobes. The youngest sex-organ develops near the meristematic zone. The type of prothallus is short lived. Examples: *L. cernuum*, *L. inundatum*.

Second type

This type of prothallus is subterranean (i.e., grows underground) and remains 1–8 cm below the soil surface. The prothalli are tuberous, 1–2 cm long and brownish, yellowish or even colourless. They are saprophytic and lead a heterotrophic mode of nutrition. They vary in size and shape in different species. They may be top-shaped (e.g., *L. clavatum*), conical (*L. complanatum*) or discoid (*L. volubile*). The prothallus is differentiated into two parts: upper, variously lobed or cup-shaped generative region and lower, conical basal region. The outermost layer is epidermis which is followed by a broad zone of cortex. Symbiotic mycorrhizal fungus is present in the cortex of basal part. The cortex is followed by elongated cells. The central part is occupied by storage tissue, which stores sufficient food material. The basal part bears several long rhizoids. The upper generative region has marginal meristem and bears sex-organs. The prothalli are monoecious. The antheridia develop first towards the centre whereas archegonia appear later near the margins. Examples: *L. clavatum*, *L. complanatum*, *L. annotinum*, *L. obscurum*, *L. volubile*, *L. serratum* etc.

Third type

This type is represented by prothalli of epiphytic species (Example - *L. phlegmaria*). The prothalli are subterranean and grow below the surface of humus on tree trunks or their branches. They are colourless, saprophytic and lead heterotrophic mode of nutrition. Each-prothallus is cylindrical, tuberous, approximately 2 mm in diameter, branched monopodially and irregularly, develops several rhizoids, which act as absorbing organs. The branches may be 1–6 mm in diameter, The sex-organs (antheridia and archegonia) develop on the upper surface of branches. The antheridia develop first whereas the archegonia appear late and they are always intermingled with paraphyses. The endophytic fungus (mycorrhiza) is present in the central cells, which also bear reserve food material in the form of oil. The branches may also develop multicellular gemmae, which serve as means of vegetative reproduction. Example: *L. phlegmaria*, *L. billardi* etc.

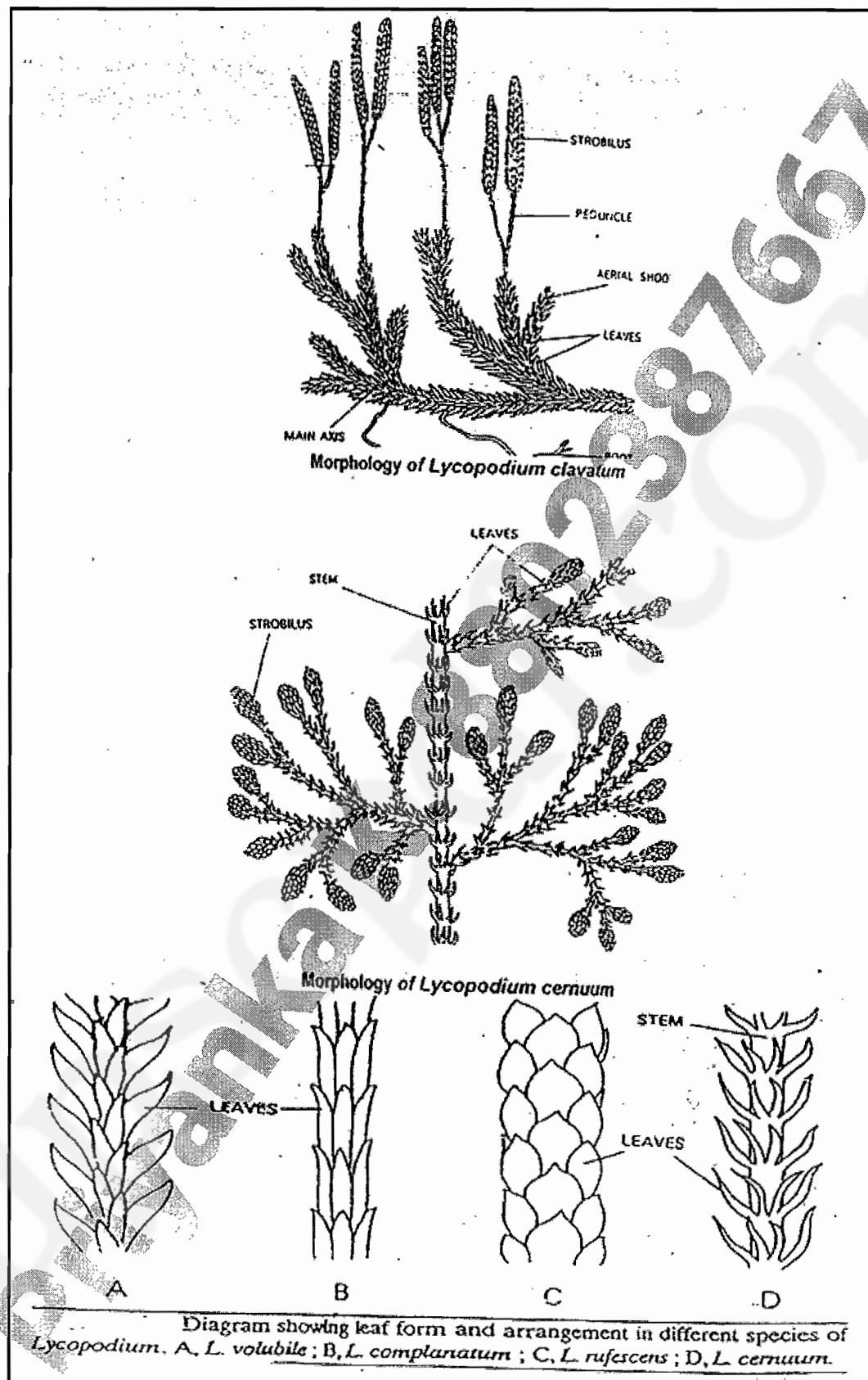
The prothalli of *L. selago* vary in their form and structure under different conditions of growth. In case the spores germinate soon after their liberation over the surface of substratum, they produce short, upright (erect), green and photosynthetic prothalli comparable to first type. If the spores are buried deep into the soil they produce subterranean, cylindrical or flat, colourless and saprophytic prothalli comparable to second type. The aerial prothalli bear several cylindrical branches comparable to third type. Thus, the prothallus of *L. selago* can be regarded as intermediate type.

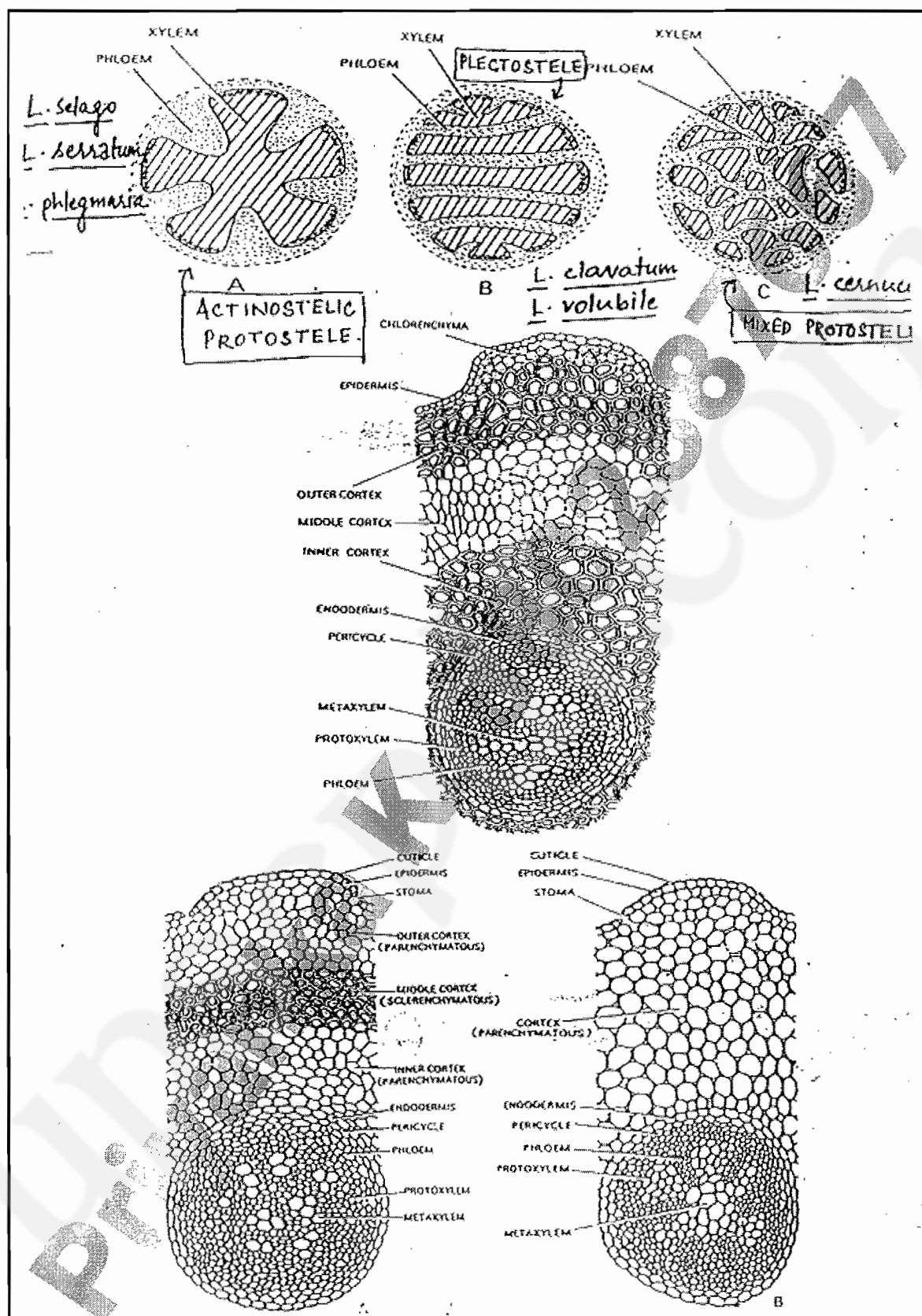
Sex-organs

The prothalli of *Lycopodium* are monoecious (bisexual), i.e., produce antheridia and archegonia on the same prothallus (gametophyte). The antheridia develop earlier than the archegonia (i.e., **protandrous**).

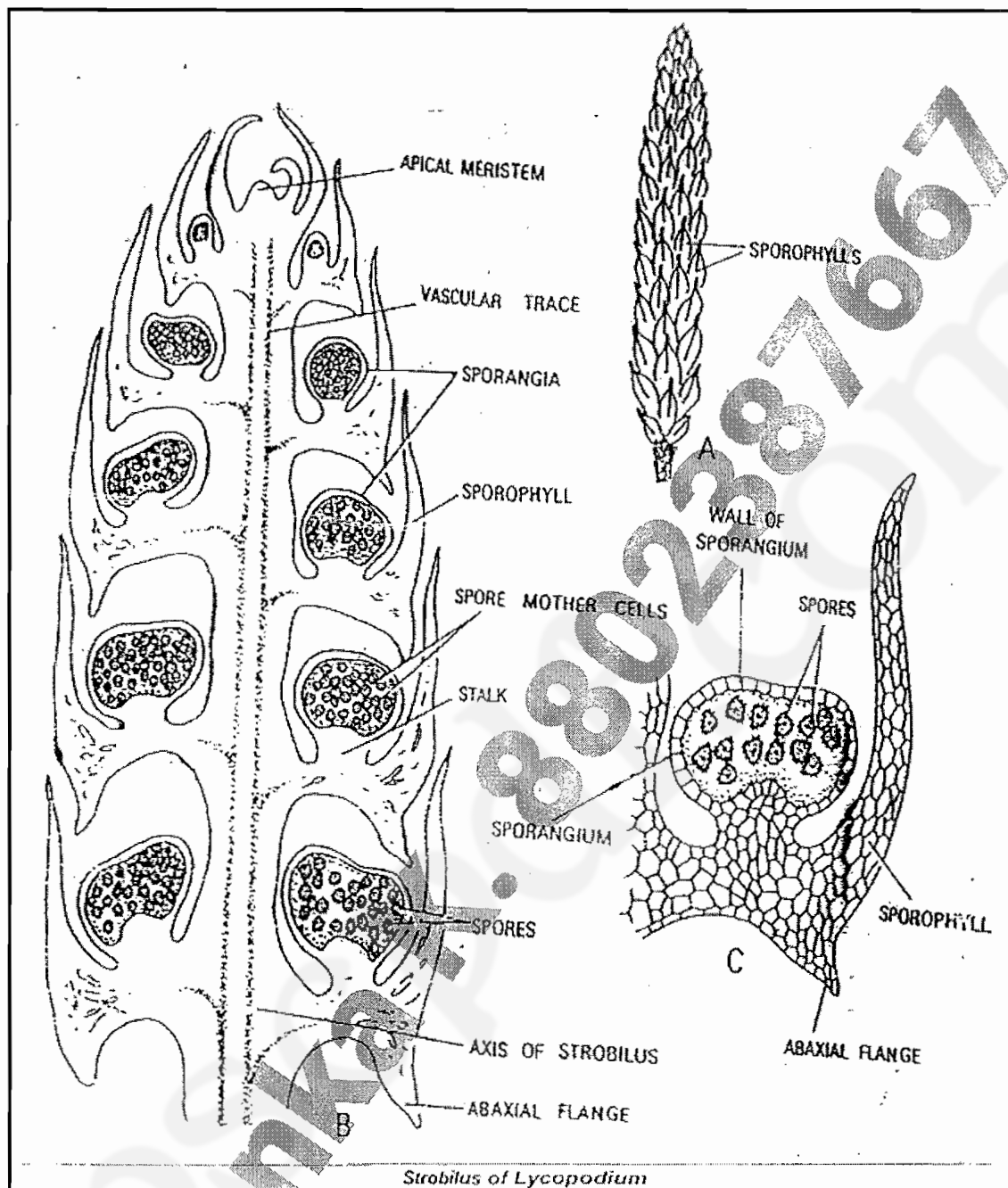
Economic importance of *Lycopodium*

1. The plants of *Lycopodium* are beautiful and therefore, used for decorative purposes. *L. obscurum*, also known as "Christmas Greens", is used in Christmas wreaths and other decorations.
2. *L. volubile* is used for table decorations.
3. Dust like spores of *Lycopodium clavatum* are used in pharmacy as water repellent and protecting dusting powder for soft and tender skin.
4. The spores of *L. inundatum* yield a high amount of oil, which are used as cover for pills.
5. Plants of *Lycopodium* have medicinal value. Their extracts were used as kidney stimulant. It is also used in homoeopathic system of medicine as the name *Lycopodium*.
6. The spores *Lycopodium* are highly inflammable and used in flares, fireworks and stage lightening.





Stem anatomy of Lycopodium species



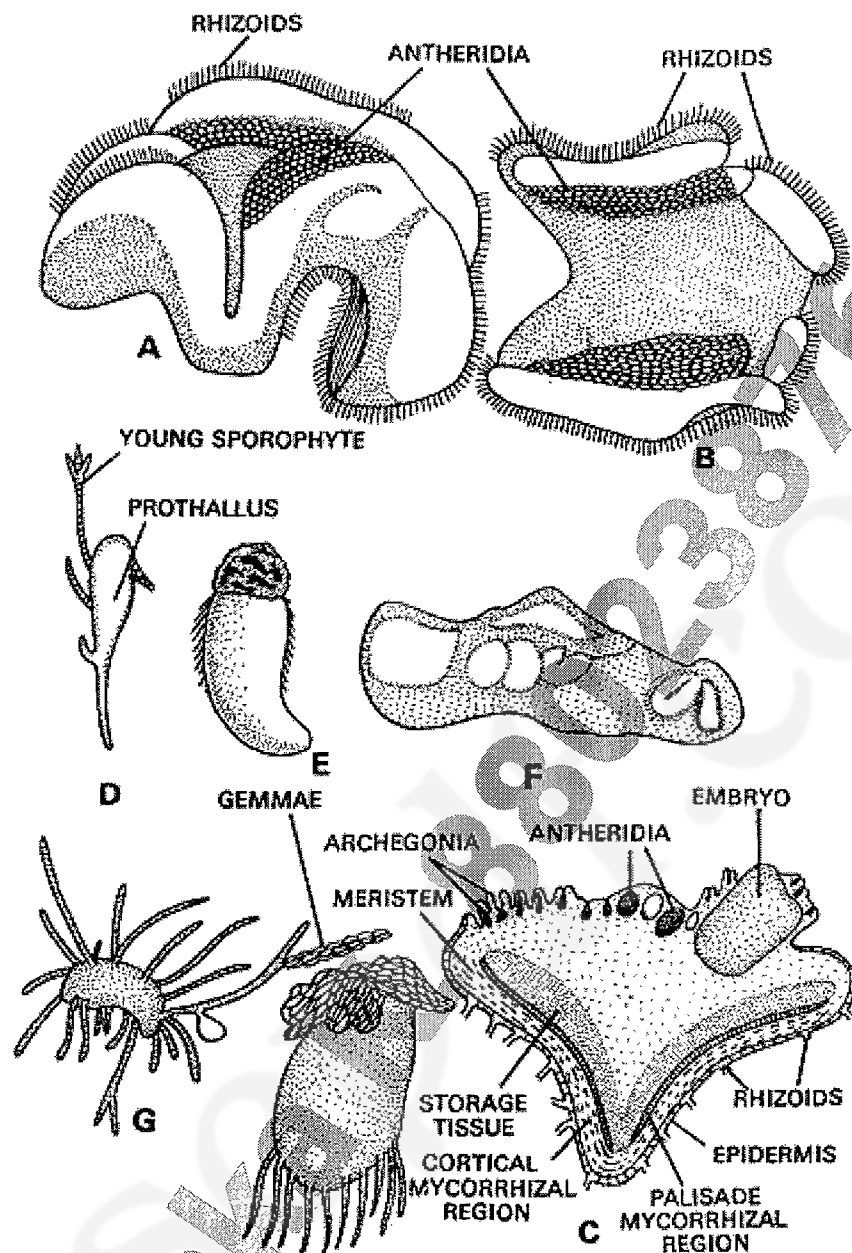


Fig. 27.16. *Lycopodium*: Different shapes of prothalli in various species. A-B, side and top aspects of mature prothallus of *Lycopodium clavatum* showing numerous antheridia and rhizoids; C, vertical section through the prothallus of *L. clavatum* showing mycorrhizal zone and reproductive organs; D, prothallus of *L. selago* with young sporophyte; E, *L. complanatum*; F, *L. annotinum*; G, *L. phlegmaria*; H, *L. cernuum*.

Figure: Different types of prothalli

Selaginella

The genus *Selaginella* includes more than 700 species out of which about 70 reported from India. They are distributed mainly in tropical and sub-tropical regions of the world, where they grow in damp and poorly illuminated forest floors. Some species grow on tree trunks and branches as epiphytes (e.g. *S. oregano*). Some are commonly cultivate in green houses as ornamentals (e.g., *S. Kraussiana*, *S. adunca*, etc.)

The plant body

The species of *Selaginella* vary considerably in the size, nature and form of plant body. Most of them are perennial herbs, but a few are annuals (e.g. *S. pygmaea*, *S. gracillima*). The plants vary in size ranging from few centimeters to several meters.

Many species have prostrate and dorsiventral plant body (*S. Kraussiana*), while others may be erect and radial (*S. selaginoides*), sub-erect (*S. trachyphylla*) or rarely scandent (*S. willdenovii*). *S. alligans* is a climber and climbs with the help of pads developed at the end of rhizophores.

On the basis of different forms and structure, the species of *Selaginella* have been divided into two sections—Homoeophyllum [with only one type of leaves] and Heterophyllum [with different types of leaves] (Hieronymus, 1900).

Stem: The stem may be erect (*S. selaginoides*), prostrate and dorsiventral (*S. chrysorrhizos*), sub-erect scandent (*S. kraussiana*) or pendent (*S. oregano*). The stems are commonly much branched (rarely unbranched), the branches and sub-branches often grow in the same plane. The branching is dichotomous but later on becomes monopodial.

Leaves: The leaves are small (microphyllous), simple, sessile and lanceolate or ovate in shape. Each leaf has a distinct unbranched midrib. They are green, thin and delicate (except in some xerophytic species where they are thick and rigid). The margin of leaves may be entire or serrate and the apex is acute.

In the species of section Homoeophyllum, all the leaves are of only one type i.e., isophyllous and arranged spirally on the stem. In Heterophyllum section, the leaves are anisophyllous (heterophyllous) i.e., they are of two kinds (dimorphic). The two rows of larger ventral leaves and two rows of smaller dorsal leaves form four vertical rows attached laterally on the dorsiventral stem. The larger and smaller leaves alternate with each other. The arrangement of leaves on stem (phyllotaxy) is opposite decussate in all species having dorsiventral symmetry. The larger leaves form pair with smaller leaves (i.e. the pairs are unequal).

All the species are ligulate i.e., Possess tongue like ligules at the base of each leaf on its upper surface a characteristic feature of Ligulopsida)

Ligule: The ligule is small tongue-shaped outgrowth present at the base of each young leaf on its adaxial side. In mature leaves it withers away and disappears. Each ligule is differentiated into two parts—glossopodium and the body of ligule. The glossopodium is basal hemispherical portion embedded in a cup-shaped glossopodial sheath. The body may be tongue-shaped, wedge-shaped, lobed or lanceolate. The function of ligule is not well known. Many workers consider it as a secretory organ that secretes water or mucilage and keeps sporangium and the young leaf wet. It may be considered as a protective organ.

Rhizophore: In many dorsiventral species of *Selaginella* several long, colorless, prop-like structures originate from the points of dichotomy of stem. These are called rhizophores. It originates from angle meristem present between the two branches of stem and grows downward i.e., +vely geotropic. It is long, cylindrical, leafless structure without root hairs and root cap. It develops adventitious roots at its terminal end. In most of the cases (e.g., *S. Kraussiana*) only one rhizophore arises from the point of dichotomy, but in few cases (e.g., *S. martensii*) two rhizophores arise from each dichotomy. However, out of two only one goes down into the soil and bear roots.

Root: The roots are mainly adventitious. The primary root is very short-lived. The adventitious roots may arise directly from the stem (e.g., *S. umbrosa*), from the swollen bases of hypocotyls (e.g., *S. chrysocaulis*) or from the apices of rhizophores (e.g. *S. Kraussiana*)— They are endogenous in origin and are dichotomously branched.

Internal features of stem

In general, the internal structure (anatomy) of stem, in all the species of *Selaginella*, follow the same pattern. However, a considerable variation can be observed in their stellar system – some are monostelic, some are disteic while a few are polystelic. A brief account of internal structure of stem, as seen in transverse section, is given below. The outline of section is wavy. It is differentiated into epidermis, cortex and stele.

Epidermis: The outmost single layered epidermis consists of thick walled, fibre-like prosenchymatous cells. It is thickly cuticularized. The stomata are absent.

Cortex: The cortex is multilayered. The outer region of cortex, in most of the species, consists of a few layered (usually 2-4 layered) thick-walled, sclerenchymatous hypodermis whereas the rest of cortex consists of thin walled parenchymatous cells. In some cases, whole of the cortex consists of thin-walled, green angular parenchymatous cells. In some xerophytic species (e.g., *S. rupestris*), the sclerenchymatous zone is much broad.

Endodermis and air-spaces: In the beginning the endodermis is single layered which surrounds the stele. Later on, the cells of endodermis elongate in radial direction and separate with each other, thus forming broad air-spaces between them. The cross-section of *Selaginella* stem (except the stems of *S. lepidophylla*), therefore, shows a zone of air-spaces radially traversed by trabeculae (modified endodermal cells). The trabeculae possess casparian thickenings, just like those possessed by endodermal cells. The trabeculae are unicellular but may become multi-cellular filamentous due to formation of tangential walls.

Stele or the vascular region: Depending upon the number of steles, the stem may be monostelic, distelic or polystelic. The following variations in the stelar organization occur in different species of *Selaginella* or even in the same species under different conditions –

1. Single flattened or ribbon-like stele (monostele) – occurs in dorsiventral stems of *S. chrysocaulos*.
2. Single cylindrical stele (monostele) with polyarch condition – occurs in erect stems of *S. selaginoides*.
3. Single-cylindrical stele (monostele) with endarch condition of xylem – occurs in fruiting stems of *S. selaginoides*.
4. Two steles (distelic condition) running parallel and joining at the points of dichotomy – occur in *S. Kraussiana*.
5. Many flattened or ribbon-like steles (polystelic conditions) – occurs in *S. willdenovii*.

The stellar organization of *S. Kraussiana* (a common garden species) is given below:

The stems possess two steles (distele) running parallel and joined at the points of dichotomy (branching of stem). Each stele has its own peripheral air chambers traversed by trabeculated endodermis. Each stele is a protostele (i.e., a central solid core of xylem is surrounded by phloem and pericycle). Pericycle is single layered. It is followed by phloem consisting of parenchyma and sieve cells. The companion cells are absent. The centre of stele is occupied by xylem. It is diarch and exarch. The metaxylem occupies central position whereas protoxylem lies towards periphery. The metaxylem is composed of scalariform tracheids whereas protoxylem has annular and spiral tracheids.

A few species of *Selaginella* (e.g. *S. rupestris*, *S. oregana* etc.) are remarkable in having true vessels in their xylem.

Reproduction by spores

The sporophytic plant body produces haploid spores which serve as the means of asexual reproduction. All the species of *Selaginella* are heterosporous, i.e., produce two different kinds of spores – (i) the microspores and (ii) the megaspores. The microspores are smaller in size as compared to megaspores. These spores germinate to produce gametophytes. The microspore germinates to produce male gametophyte and the megaspore germinates to produce female gametophyte.

The spore producing organs the strobilus or cone

The spores are produced inside the sporangia borne in the axils of sporophylls. The microspores are produced in large numbers inside the microsporangia and the megaspores are produced in limited small numbers (about 1-12) inside the mega sporangia. The micro sporangia and mega sporangia are borne singly in the axils of microsporophylls and megasporophylls respectively. The initial development of each kind of sporangium (i.e., microsporangium and mega sporangium) is the same adaxial eusporangiate type, i.e., develops from a group of initial cells between the ligule and the axis.

These sporophylls are located into a definite compact (or sometimes loose) body called strobilus or cone. It is also called as sporangiferous spike.

A strobilus or cone of *Selaginella* consists of a central axis on which the microsporophylls and megasporophylls are arranged. Both micro and megasporophylls are like ordinary vegetative leaves, but differ in size and shape. Each sporophyll possesses a ligule. The sporophylls, in a strobilus, may be isophyllous (e.g., *S. setaginoides*) or anisophyllous (e.g., *S. Kraussiana*). They may be arranged spirally or opposite decussate.

The strobili vary in size and shape in different species. In most of the species they are long, quadrangular (four ranked) in shape and tapering towards the apex. In a few cases they are cylindrical (e.g., *S.*

setaginoides) or dorsiventral. A great variation also exists in the distribution and location of micro- and megasporophylls in the different regions of the strobilus. In the basis of their distribution, following categories can be made-

1. In most of the species the mega sporangia are located in the basal portion mid the micro sporangia in the upper portion of the strobilus (e.g., *S. chrysocaulos*, *S. Rupestris*, *S. selaginoides*, etc.).
2. In some species, one side of the strobilus bears micro sporangia and the other side bears mega sporangia (e.g., *S. oregano*, *S. inaequalifolia*, *S. pilifera*).
3. Only one megasporangium, lies at the base and rest all are micro sporangia in the strobilus of *S. Kraussiana*.
4. The mega sporangia and micro sporangia are irregularly located along the entire length of the strobilus (e.g., *S. manensii*).
5. The micro sporangia and mega sporangia are borne in separate strobili, but on the same plant (e.g., *S. gracilis*).

Heterospory in *Selaginella*

All the species of *Selaginella* are heterosporous i.e., they produce two different kinds of spores in each plant. These spores differ with each other mainly in their size and function. The smaller ones, called microspores, are produced in large numbers in micro sporangia and the larger ones, called megaspore, are produced in smaller numbers inside the mega sporangia. The initial development of each kind of spores, inside the sporangia, is the same but the difference starts with the formation of spore mother cells. In the micro sporangia all the microspore mother cells undergo meiosis and form the microspores. Therefore, they are produced in large numbers and their size also remains small (ranges between 0.015 to 0.05 mm in diameter). On the other hand, all the spore mother cells in a mega sporangium do not complete their development. Only one or a very few megaspore mother cells undergo meiosis and produce a very small number (usual 4) of functional megaspores. Consequently the megaspores become much larger in size ranging between 1 to 5 mm in diameter) and store more reserve food as compared to microspores.

The micro- and megaspores not only differ in their size but also differ in their function. Each microspore germinates in situ and produces a male gametophyte. The vegetative part of male gametophyte is represented only by a single prothallial cell. The remaining portion of male gametophyte becomes an antheridium, which produces 8 sterile jacket cells and 128-256 antherozoids. The megaspore also germinates in situ and produces a female gametophyte. The female gametophyte has a large number of vegetative cells, filled with reserve food, so that they can provide proper nourishment to the development embryo.

The degeneration of nonviable megaspore mother cells and the formation of functional megaspores in *S. sulcata* have been worked out by Pettit (1911). According to him the viable mother cells are larger (about 15 microns or more in diameter) whereas non-viable ones are smaller in size (about 7 to 10 micron in diameter). In addition, the viable ones are characterized by the aggregation of mitochondria/plastids at the prophase and contain intra-nuclear vesicle in their nuclei. The non-viable ones, on the other hand, possess lysosomes. Similarly the viable megaspores inherit the mitochondria or plastids from the mother cells whereas the non-viable ones do not. This can explain, up to some extent why certain megaspore mother cells or megaspores remain viable and others degenerate. However, Brooks (1973) indicated that the formation of mega sporangia in *Selaginella* is under the control of ethylene—a hormone that retards cell division.

Biological significance of heterospory

The most remarkable feature of heterospory is the determination of sex by sporophytic plant during sporogenesis. The homosporous pteridophytes, on the other hand, carry out the determination of sex by the gametophyte at the time of differentiation of antheridia and archegonia. Thus, the heterospory has shifted the sex determining capacity from the gametophyte to sporophyte.

In *Selaginella*, the smaller microspores are destined to produce male gametophytes and the larger megaspores to female gametophytes. The male gametophyte produces male gametes (antherozoids) whereas the female gametophyte produces archegonia and also provides nourishment to the developing embryo. It is, therefore, the megaspores are much longer and store more reserve food as compared to microspores. In addition, the spores get their nourishment from the sporophyte according to their need. This has led to the reduction of gametophytic tissue and more elaboration of sporophyte, which are well adapted to survive under varied environmental conditions prevailing on land.

The phenomenon of heterospory lead to the reduction of gametophyte. In situ germination of spores, retention of mega gametophyte in the mega sporangia and finally to the seed development.

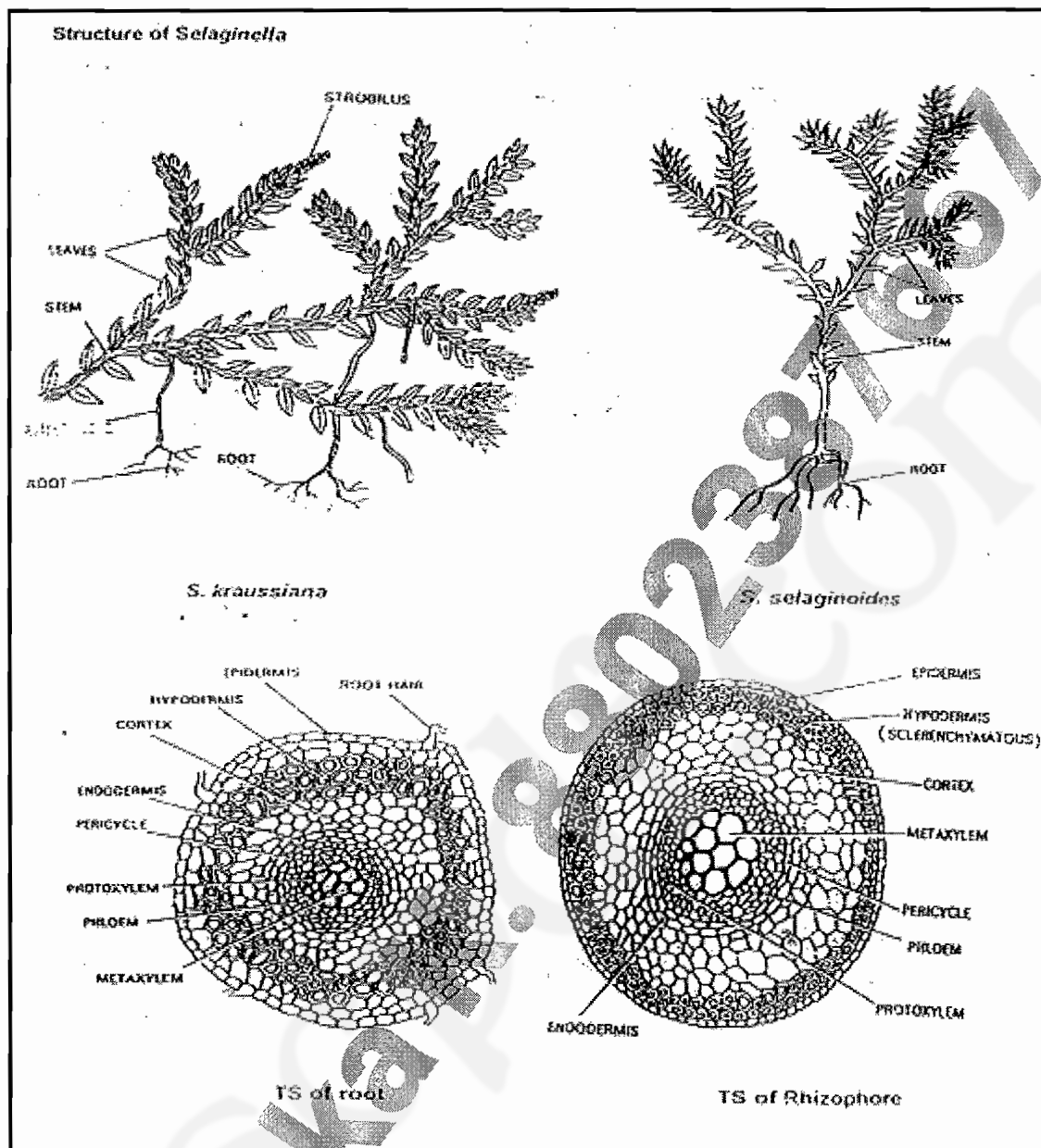
Seed habit in *Selaginella*

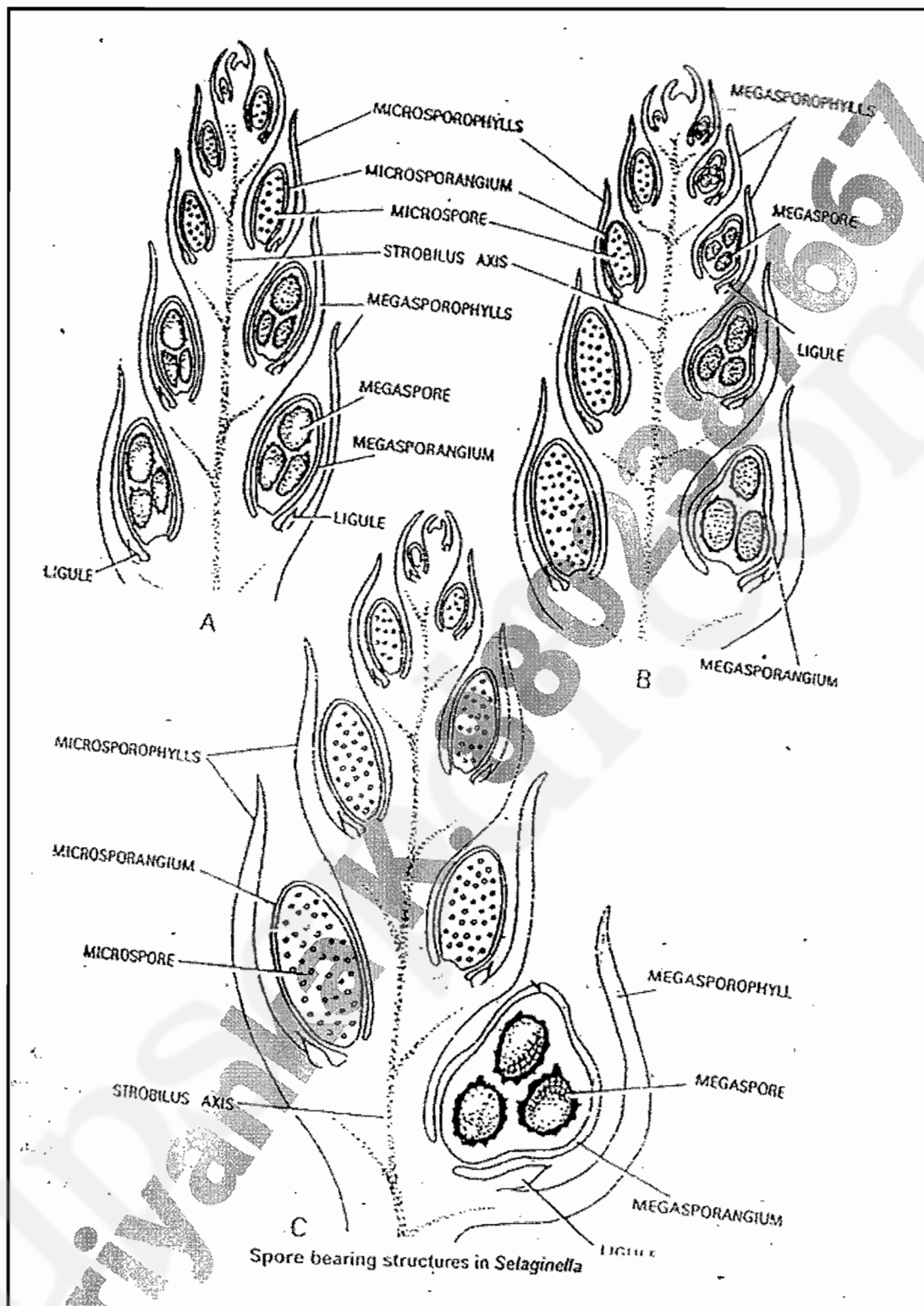
1. The first step towards a "seed habit" is a heterosporous life cycle, where two types of spore's i.e. Microspore producing male gametophyte and megaspore producing female gametophyte are found. This step has been achieved by all the species of *Selaginella*.
2. The second step is the retention of megaspore within the mega sporangium. The germination of megaspore by cell divisions within the wall of megaspore results in the formation of a 'captive female gametophyte'.

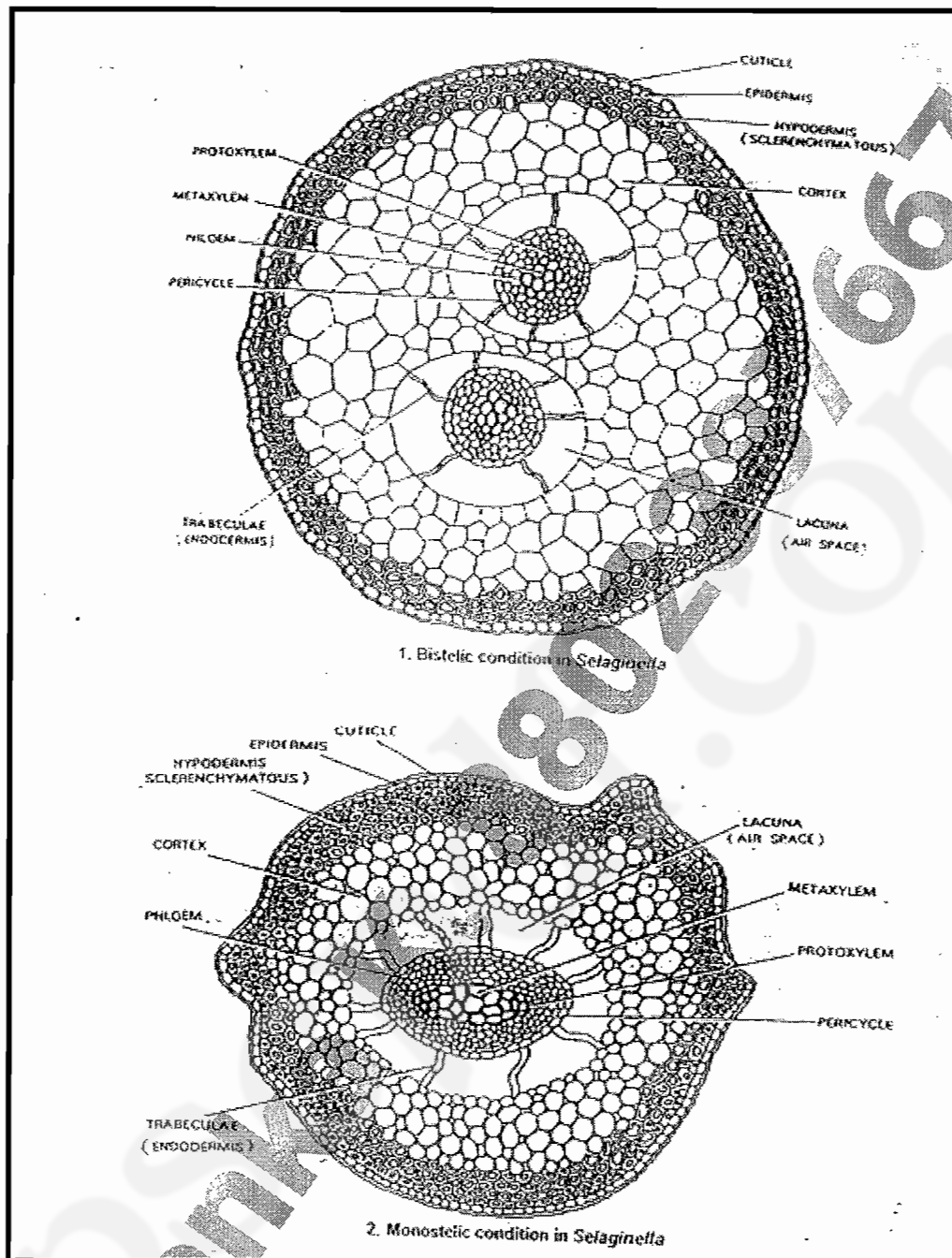
In most of the species, of *Selaginella*, the megaspore is retained within the mega sporangium. It germinates in situ and grows up to an advanced stage of female gametophyte. In *S. Kraussiana*, the first archegonial initial is set up before the semi-germinated megaspore is shed. Further advancement has been achieved by *S. rupestris*, where only one megaspore is produced within the megasporangium. It grows and produces a well developed female gametophyte, which after fertilization forms an embryo and the megaspore is still enclosed within the mega sporangium and attached the mega sporangium and attached to strobilus. The embryo develops shoot apex, cotyledons and roots. This stage can be compared with the phenomenon of vivipary that occurs in some angiosperms.

3. The third essential features are transport of sperm nuclei to the female gametophyte other than by swimming. The seed plants produce small and easily transportable male gametophytes-the pollen grains, which carry sperm nuclei to the female gametophyte.
4. The fourth step is to develop an efficient means of sperm delivery to the egg the pollen tubes). The third and fourth steps are concerned with the internal syngamy. The final step is to develop a means for the protection of embryo by a seed coat and production of some inhibitors which may cause the embryo to remain dormant until the environment conditions become favourable for germination.

In *Selaginella*, the mega sporangium can be compared with the nucellus of an ovule where the integuments (seed coats) are not found. The mega gametophyte is not permanently retained within the mega sporangium and the embryo does not have any period of dormancy. Thus, the genus *Selaginella* can be treated as base for setting some primary requirements towards the 'seed habit'. These features are valued very remarkably and significantly while studying the evolution of "seed".







Isoetes

The genus is represented by about 70-spp. distributed across the globe. Some common Indian species include *I. coromandelina*, *I. unilocularis* and *I. dixitii*. The name Quilworts for this genus is popular among the botanists and the common people alike in the Americas. Merilyn's grass is a name commonly used in Europe.

Most of the species are submerged, either wholly or partially. Only a few of them are truly terrestrial.

Morphology

Like all other pteridophytes, the dominant and ecologically persistent stage of the life cycle in this genus too is the sporophyte.

The sporophyte gives the appearance of Liliaceous or Gramineous monocotyledons having crown of tightly packed linear leaves, underground stem-like 'axis' and tuft of roots.

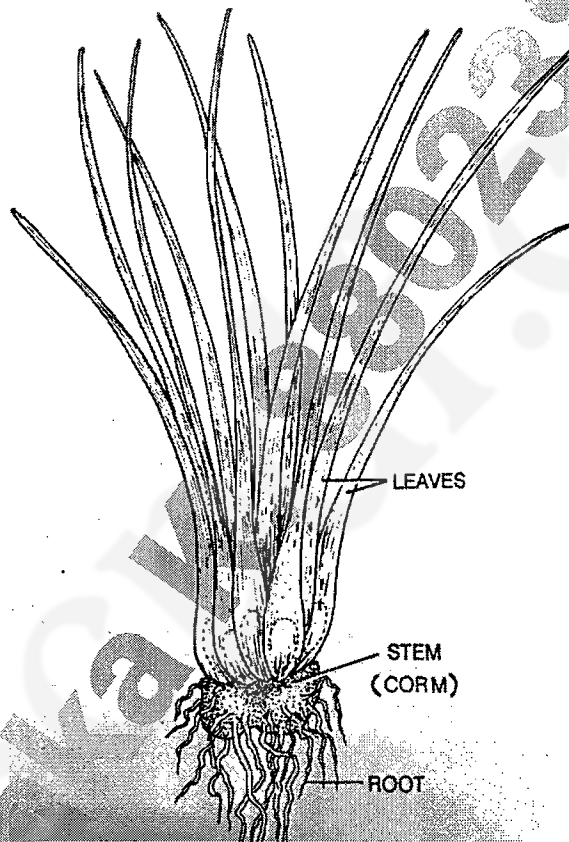


Figure: Sporophyte morphology

The axis is quite reduced, subterranean, erect structure commonly termed as 'corm'. At maturity it is a two, three or rarely, four lobed structures. The upper part of axis is covered with a dense duster of leaves and the lower part lateral roots.

A J Eames [1934] and later Barnes (1936) have used the term axis for the overall corm and stem for the upper leaf-bearing part and rhizomorph for the lower root bearing part of the axis.

The shoot apex is hidden in a depression formed by the tightly overlapping leaves on the upper part of the axis.

The true morphological nature of the corm in *Isoetes* spp is a well debated question. Workers like Osborn [1922] and Stewart [1947] have used the term cone axis for the upper part of the cone and stigmarian axis for the upper part of the corm and strigmarian axis for the lower root bearing part of it.

One of the characteristics feature of *Isoetes* axis is the formation of 'caps' or 'shoulders', which are the dried leaf bases and roots of the previous year, so that the sides of mature plants become very rough. These caps or shoulders are sloughed off towards the end of the growing season. This is essentially a process of secondary growth.

The leaves are microphyllous, ligulate, borne acropetally in dense clusters of up to 20 at the upper part of the axis. They are differentiated into broad overlapping spoon-shaped bases and long narrow upper parts. The lower portion of each leaf is broad, underground and lacks chlorophyll so that it is pink' or glistening white in colour. The upper linear part of each leaf is green and photosynthetic.

The mature fertile leaves that bear sporangia are sporophylls. In fact, each foliage leaf is a potential sporophyll. The sporangium is attached on the adaxial side near the base of each leaf.

Each leaf is characterized by the presence of a ligule and a velum. The ligule is triangular, cordate or bifid in shape, colourless, and attached to the tissue of the leaf with the help of lobed structure called glossopodium. The glossopodium is deeply embedded in the leaf tissue and consists of a transverse bar and a pad that connects two side arms. Functionally the ligule is regarded as absorbing or secretory structure that secretes water or mucilage to keep the leaf and sporangia moist.

Velum is a small flap-like structure present between the sporangium and the ligule. It originates through the periclinal divisions in surface cells of leaf below the ligule and grows into a lobed structure. The size, shape and number of lobes of velum vary in different species of Isoetes. It is single lobed in *I. Coromandelina*, *I. Malinverniana*, *I. Indica* and *I. Asiatica* whereas two-lobed in *I. Sahyadrii* and *I. sampathkumarini*. The velum is absent in some species.

Roots: The first embryonic root arises laterally and exogenously. The other roots arise endogenously in acropetal succession. The root is dichotomously branched and possesses numerous roots hairs.

Anatomy of the corm

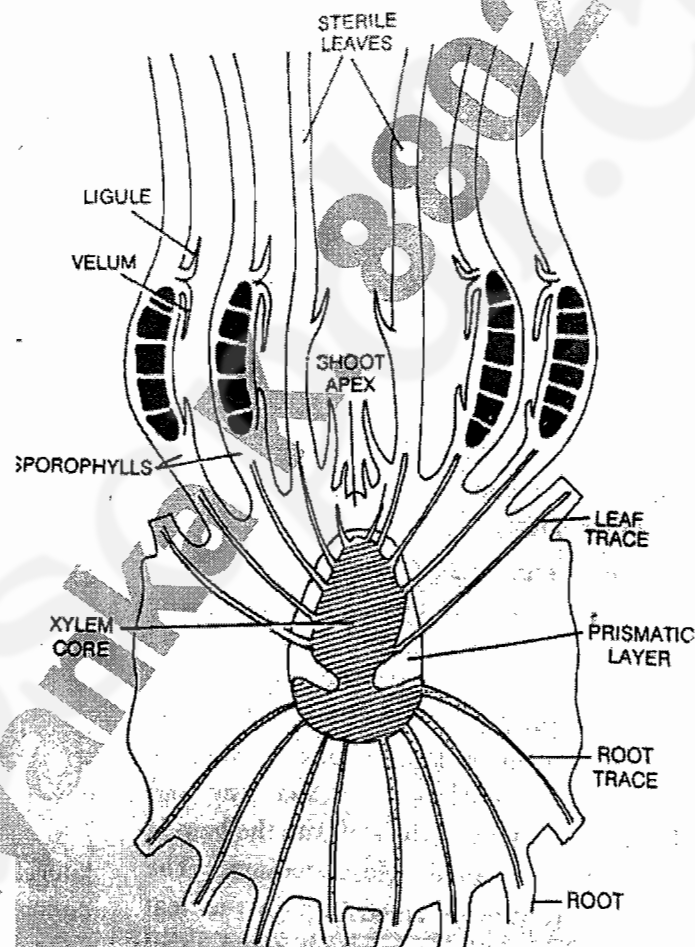


Figure: LS through the corm

The primary structures of axis consist of a central core of primary xylem surrounded by primary phloem, primary cortex and epidermis. The cortex is traversed by a large number of leaf traces in the upper part of axis and by root primordia in the lower part. The outer cells of cortex are chlorenchymatous whereas the inner cells are parenchymatous with many intercellular spaces. The cells of cortex possess starch grains. The stele is protostele in which the central solid core of xylem (consisting of tracheids and parenchyma) is surrounded by phloem. There is no differentiation of protoxylem and metaxylem.

The complete vascular cylinder of the axis can be studied best if a mature plant is cut longitudinally in the plane of the basal groove or furrow. The central xylem core is anchor-shaped (or inverted T-shaped), i.e., cylindrical in the upper part and horizontal with upturned arms in the lower part. The roots and root primordial are seen arising from below the horizontal xylem core.

The secondary growth is the most characteristic feature of *Isoetes* axis. The cambium is extra-stelar originating outside the primary phloem. It is made up of two distinct parts (i) a lateral meristem and (ii) a basal meristem. The lateral meristem (storied cambium) gives rise to prismatic layer (vascular tissue) towards inner side (centripetally) and secondary cortical tissue towards outer side (centrifugally). The prismatic layer consists of sieve elements alternating with layers of parenchyma, a few cells of which differentiate as tracheids. The secondary cortex, consisting of parenchymatous cells, is added to regularly and the outer part sloughs off from the shoulders of the corm. The basal meristem is located on the lower biconvex surface of the vascular cylinder and remains continuous with the lateral meristem. It gives rise to secondary xylem and parenchyma towards the basal vascular cylinder and parenchymatous cells towards the periphery. The cells of secondary cortex store food material.

Leaf Anatomy

Transverse section of a mature leaf shows quadrangular outline with two expanded lateral wings on the lower side. The outer most layer is epidermis consisting of compactly arranged cells. The terrestrial and amphibious species possess stomata on exposed surface. The submerged species lack stomata. The outer surface of epidermis is covered by a thin layer of cuticle.

The epidermis is followed by undifferentiated mesophyll consisting of parenchymatous cells with many chloroplasts and starch grains. The upper portion of a leaf is traversed longitudinally by four large air chambers (lacunae) that are partitioned by several layered thick diaphragms. There is a single central collateral vascular bundle that runs throughout the length of the leaf.

Root anatomy

A transverse section of a mature functioning root shows outermost single layered epidermis consisting of thin walled cells. It is followed by 4-8 layered parenchymatous cylindrical cortex which surrounds a large air cavity and a vascular cylinder. The large air cavity is formed by the breakdown of cortical cells. The vascular cylinder is surrounded by single layered endodermis with casparian strips. It is attached to the cortex on one side of the central cavity. The side is collateral having xylem on one side and phloem on the other side. The phloem is oriented towards the central cavity.

Reproduction by spores

All the species of *Isoetes* are heterosporous, i.e., produce two different kinds of spores – (i) the microspores and (ii) the megaspores. The microspores are smaller in size as compared to megaspores. These spores germinate to produce gametophytes. The microspore germinates to produce male gametophyte and the megaspore germinates to produce female gametophyte.

Sporophylls and Sporangia

The spores are produced inside the sporangia borne on the sporophylls. The micro sporangia are borne on the microsporophylls and the mega sporangia are borne on the megasporophylls. The sporangia are borne singly at the base of sporophyll towards the adaxial side.

In *Isoetes*, there are no organized strobili or cone. The sporophylls are directly attached to the axis in close spirals. In most of the species, the arrangement of microsporophylls and megasporophylls is not regular. However, in some species (e.g. *I. lacustris*) the megasporophylls are restricted to the periphery and the microsporophylls occupy the central part of the axis. A few sterile leaves are found in the central part. Generally the new sporophylls develop in every growing season and by this time the old sporophylls of previous season fall off.

The sporangium (either micro-or megasporangium) is borne singly at the expanded and thick base of the sporophyll sunken on the adaxial surface. Each sporangium is sessile, large, 4-10 mm in length and almost oblong-rounded in shape. A tongue-shaped ligule is present just above the sporangium. A small protective flap-like velum is also present in between the sporangium and the ligule which partly or completely covers the sporangium. Each sporangium is internally traversed by ribbon or plate-like trabeculae, which do to divide the sporogenous mass completely into separate compartments or locules.

The sporangial development is eusporangiate type i.e., develop from a group of initial cells. The sporangium and the velum both start developing very early in the ontogeny of a leaf. A group of superficial initial cells below the ligule, divide by periclinal divisions to give rise to velum and sporangium. It is the velums that develop first and is followed by growth of the sporangial.

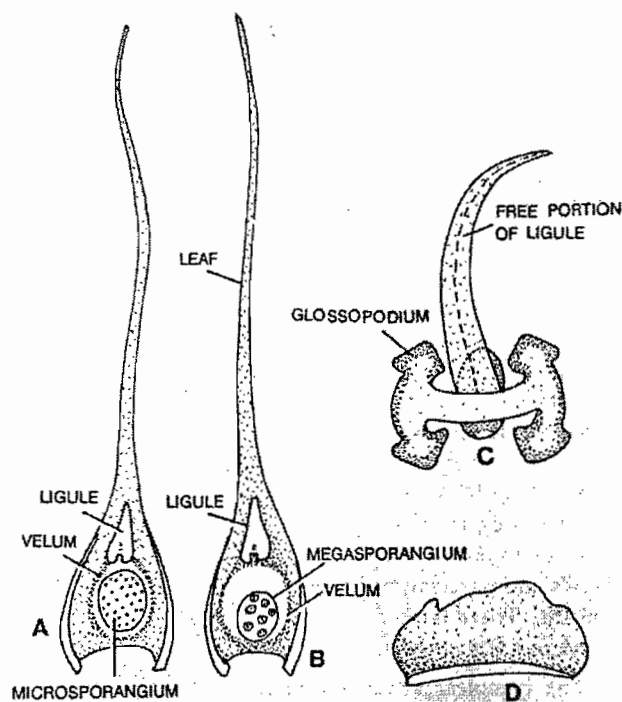


Figure: Sporophyll morphology

In case of developing microsporangium, the microspore mother cells (microsporocytes) separate from each other from the irregular groups. Each of these cells divides by meiosis to produce spore tetrads. There is very large number (ranging from 3,00,000 to 1,000,000) of bifacial microspores in each microsporangium.

In case of developing megasporangium, all the spore mother cells do not participate in the formation of megaspores. Some of them degenerate and resorbed. The remaining megaspore mother cells greatly enlarge and divide by meiosis to form approximately 100 to 300 tetrahedral megaspores. The size of mature megaspores ranges between 200 to 900 micrometers in diameter. They may be white, grey or black in colour and may be smooth or have a distinct ornamentation.

Dehiscence of sporangium: In Isoetes. The sporangia are indehiscent. The spores are liberated by decay of sporophyll in winter season. In some species, where the corm is entirely buried, the sporophylls of previous season are forced up by the expansion of mucilage cells at the base of the sporophylls. The spores are dispensed by wind.

The gametophytic generation

The gametophytic generation begins with the production of spores. All the species of Isoetes are heterosporous.

Each microspore germinates to produce male gametophyte and each megaspore germinates to produce female gametophyte.

Male Gametophytes are comparatively much smaller structures. They are completely endosporic, nine celled structures.

It is developed by a microspore. The latter divides unequally to produce a small prothallial cell and a large antheridial cell. A series of divisions divide the antheridial cell into four jacket cells enclosing a primary sporogenous cell. The latter divides mitotically to produce four sperm cells, each one of which develops into an antherozoid, which is spirally coiled, ribbon formed, uninucleate and multiflagellate structure bearing a pronounced terminal vesicle. According to Parihar (1977) the male gametophyte in Isoetes is more reduced than any other pteridophyte.

Female gametophyte [Mega gametophyte] is also initially endosporic like the male gametophyte but in the later stages a portion of the megaspore wall gets exposed at the triradiate ridge. Initiation of the female gametophyte from the megaspore takes place by a series of free -nuclear divisions.

A mature female gametophyte is a small, yet multi-cellular body. Archegonia start developing in the upper exposed portion of the gametophyte.

A mature archegonium consists of a small neck of four tiers of four cells each. It also contains a binucleate neck canal cell, a ventral canal cell and an egg.

Details of fertilization in Isoetes are not clearly known but it certainly takes place.

Fertilization leads to the formation of a zygote, which after embryogenesis gives rise to the sporophytic generation.

Equisetum

Equisetum, which is also called Horsetail, has a very low diversity as it has only 18 species. It is distributed across the world except Australia & New Zealand.

Based on the fossil record we know that *Equisetum* was present in the Carboniferous & has changed little over time. Related plants once formed the dominant vegetation over a vast area of the planet. There are many extinct species including Trees.

Some of the common Indian species of this genus are *E. arvense*, *E. diffusum*, *E. debile* and *E. ramosissimum*. *E. arvense* is a widely occurring species.

Habitat

Various species of *Equisetum* grow in a variety of habitats. Certain species occur in ponds in marshy conditions (*E. palustre*), some grow in damp shady places (e.g. *E. pratense*), and while still other species occur in well-exposed dry conditions as well as in open grass fields (*E. arvense*). *E. arvense* is commonly called field horsetail.

Equisetum species are used as ecological indicators, especially to indicate the mineral contents of the soil. *Equisetum* species accumulate minerals, including gold, in their bodies (up to 4.5 oz. per ton), and can therefore well be used to determine the presence of minerals in the soil.

Structure

The size of the sporophytic plant in most of the species ranges between 15 and 60 cm in height. *E. ramosissimum*, an Indian species, may attain a height of 4 meters. The stems remain only approximately 2 cm thick, and therefore the plants depend on the other surrounding vegetation for their support.

All the *Equisetum* species are well-branched perennial herbs, and in all of them, there is a horizontal underground rhizome. Many roots arise towards lower side and many erect aerial shoots towards upper side of the rhizome. In species such as *E. arvense*, *E. telmateia* and many others, the aerial shoots or stems branch profusely (Fig. 1).

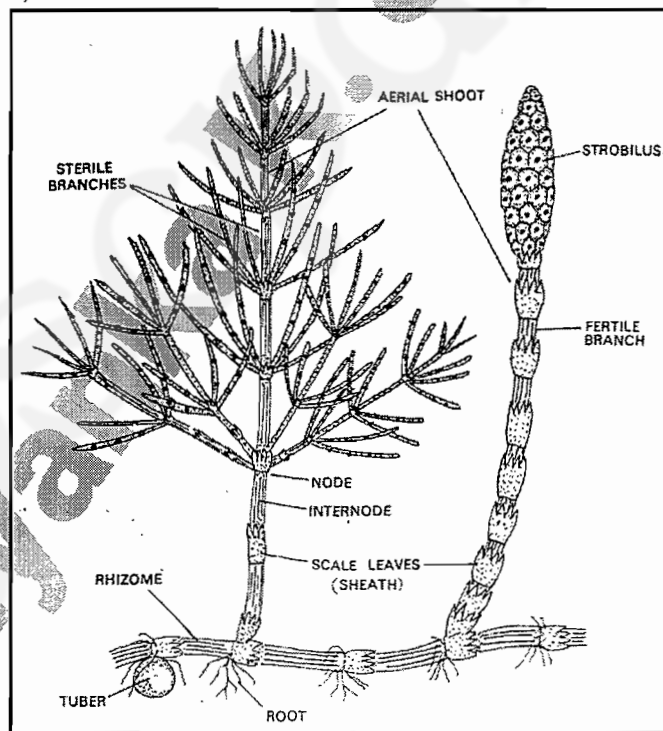


Figure 1: *Equisetum arvense*

The rhizome is long, creeping, well branched and divisible into nodes and internodes. The rhizome as well as aerial shoots (sterile as well as fertile) are articulated or jointed. The stem on its surface contains

longitudinal ridges. The number of ridges of the internodes corresponds with that of the number of leaves. A ridge lies in a straight line with that of a leaf of next upper node.

In general, the aerial shoots are of two types:

- **Sterile:** The sterile shoots are well branched, green and photosynthetic.
- **Fertile:** Generally, the fertile shoots are unbranched, colourless, bear cones at their apices, and are reproductive in function (Fig. 1).

On the node of aerial shoot are present many scaly leaves. The leaves are scaly, minute, thin, uninerved, present in the form of a whorl over the node of rhizome as well as aerial shoots. These scaly leaves are more or less united laterally with each other to form a sheath on the node. A branch primordium, alternating with each of the leaf, is present on the node.

From the nodes of aerial sterile shoots, arise two types of branches, generally in whorls. Some are long, well branched, unlimited in growth and contain the same structure as the main axis of the aerial sterile shoots. Such a pattern of branching can be observed in *E. arvense*. Others are short, unbranched and limited in growth but also bear nodes and internodes.

Roots: The roots develop from the node of rhizome or from the base of the stem. They are long, slender, well branched and adventitious. They arise endogenously from the base of lateral branches or dormant branch buds. These adventitious roots survive for several years but a definite persistent root system is not established in *Equisetum*.

Anatomy of the shoot and its adaptive features

Sporne noted about *Equisetum* shoot that it shows "an interesting association of xeromorphic and hydromorphic characters, together with a vascular system which is without parallel in the plant kingdom today."

The entire structure is wavy in outline because of the presence of ridges and grooves. In a cross-section, the internode of aerial sterile shoot is divisible into:

1. **Epidermis**
2. A well-developed broad cortex
3. A central stele
4. A large central pith cavity

Epidermis is the outermost layer of the internode, consisting of elongated cells with a deposit of silica in their outer and lateral walls. Because of the presence of silica, the stem appears hard and rough to the touch. The continuity of epidermis is broken by sunken stomata (a xerophytic character) present on the sides of grooves.

The stomatal apparatus consists of an outer pair of subsidiary or accessory cells and an inner pair of guard cells. The guard cells are completely covered by subsidiary cells.

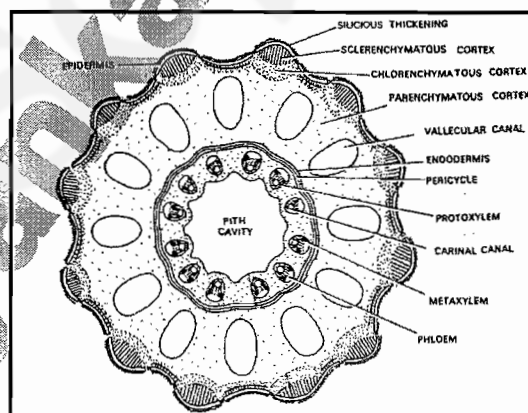


Figure 2: TS through *Equisetum* internode

Hauke (1963) has divided *Equisetum* into two subgenera based on the stomatal structure, i.e., *Equisetum* and *Hippochaete*. According to him, the subgenus *Equisetum* contains the horsetails, i.e. *E. arvense*, *E. palustre*, *E. pratense*, *E. sylvaticum* and *E. temateia*. The stomata in this subgenus are not sunken and are scattered or grouped in broad bands. However, the subgenus *Hippochaete* contains *E. giganteum*, *E. hyemale*, *E. laevigatum*, *E. myriochaetum*, *E. ramosissimum* and *E. scirpoides*, the stomata are sunken and arranged in narrow bands.

Cortex is well-developed and highly differentiated. Just inside each ridge is present a large patch of sclerenchyma followed by chlorenchymatous tissue. Chlorenchyma extends upto the epidermis in each furrow where lie the stomata. The sclerenchyma is also present inside the grooves in between chlorenchyma. The sclerenchyma is mechanical in function while chlorenchyma is photosynthetic in nature. Rest of the multilayered cortex is parenchymatous with a large air canal, called **vallecular canal**. These canals are **schizogenous** in origin. Regarding their position these canals alternate with the vascular bundles because vallecular canals are present inside the grooves while vascular bundles are situated inside the ridges.

Endodermis: Its position varies in different species. In *E. palustre* the endodermis forms a general sheath surrounding the entire stele while in *E. sylvaticum* an inner endoderm is present besides the general outer endodermal layer. The condition of *E. palustre*, is most common, and has also been reported in species such as *E. arvense*, *E. pratense* and *E. telmateia*.

Pericycle is present as a single-layered zone. In some cases, the pericycle is not very clearly demarcated.

The **vascular system** of *Equisetum* is **siphonostelic**. The vascular bundles are present below the ridges and alternate with the vallecular canals of the cortex. They are arranged in a ring. The number of the vascular bundles and vallecular canals is equal to the numbers of ridges and grooves respectively.

Each vascular bundle is conjoint, collateral, closed and consists of xylem, phloem and some parenchyma. In each vascular bundle is present a water containing cavity or canal called **carinal canal**. The xylem is 'V' - shaped, **endarch**, and consists of protoxylem and metaxylem.

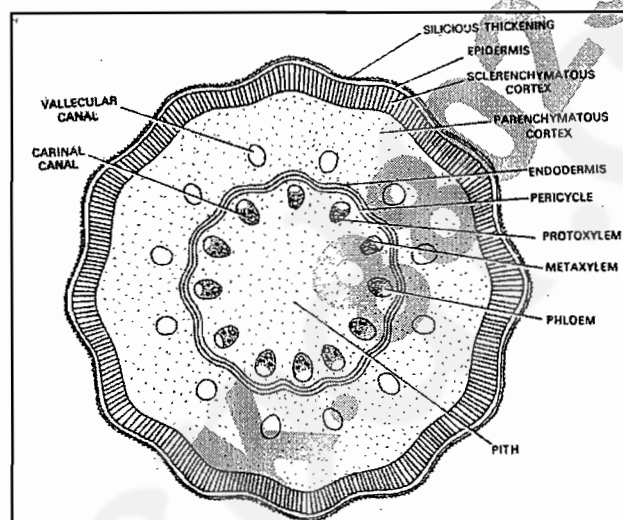


Figure 3: TS through the node of *Equisetum* stem

In young vascular bundles, the protoxylem consists of few annular and spiral tracheids. It lies opposite to the **carinal canal**, which is lysigenous in nature. Bierhorst (1958) has mentioned that the carinal canal represents a conducting channel.

The phloem in each vascular bundle is present in between two strands of metaxylem. It consists of sieve tubes and phloem parenchyma.

Pith is present in the form of pith cavity. The pith cavity is absent in *E. scirpoides*.

The stem node, depicted in Figure 3 has a solid pith structure and a continuous type of sub-epidermal structure comprising of sclerenchyma. Leaf traces and branch traces arise below the ridges and grooves, respectively and alternate to each other. Other features resemble the stem intermodal anatomy.

Xerophytic characters of aerial shoot

1. Presence of ridges and grooves
2. Thick cuticle
3. Sunken stomata
4. Presence of silica in the epidermal walls
5. Reduced and scaly leaves
6. Well-developed sclerenchyma
7. Presence of chlorenchymatous cortex which indicates the photosynthetic nature of stem

8. Presence of well-developed vascular cylinder.

Hydrophytic characters of aerial shoot include the Presence of vallecular canals, carinal canals and a well-developed pith cavity.

Anatomy of Internode of Rhizome

The rhizome resembles the aerial sterile shoot except following dissimilarities:

1. Ridges and grooves are not as prominent as in sterile aerial shoot.
2. Absence of stomata.
3. Absence of chlorenchymatous region in the cortex.
4. Poorly developed sclerenchyma.
5. Ill-developed pith cavity, which sometimes also becomes a solid structure.

Anatomy of Internode of Aerial Fertile Shoot

Except for the following few minor differences, this also resembles that of aerial sterile shoot:

1. Absence of stomata in some cases.
2. Ill-developed chlorenchymatous and sclerenchymatous regions.

Spore-Producing Organs

Spores are present in sporangia and the sporangia are borne in cones. The cones are present terminally on the main axis or sometimes on the lateral branches. Eames (1936) has mentioned that advanced species of *Equisetum* bear only one single cone present terminally on the main axis while the primitive species have few or many cones on the stem.

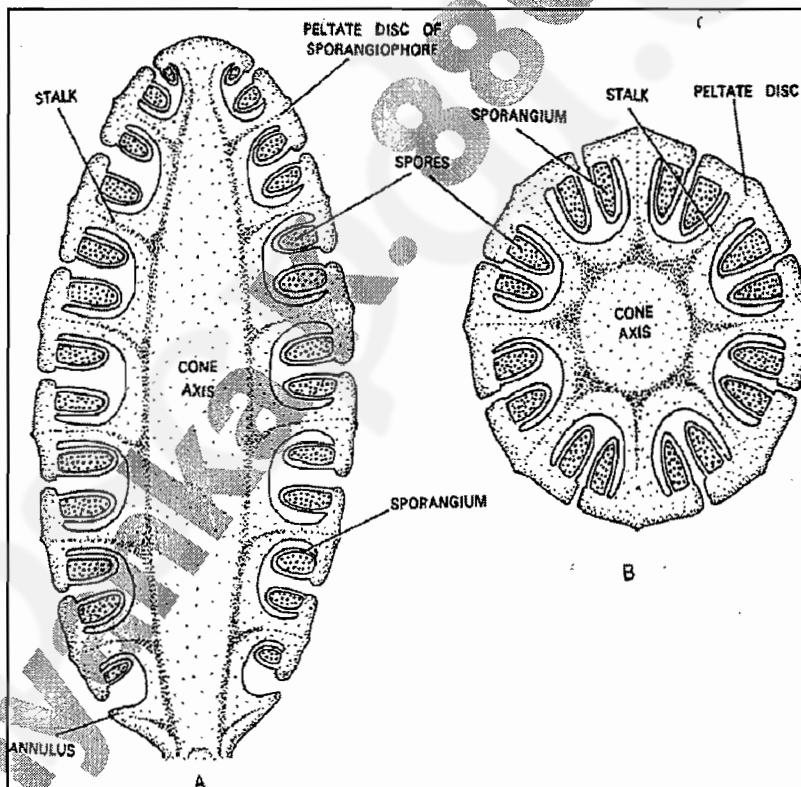


Figure 4: A. Longitudinal Section through the strobilus axis in *Equisetum*; B. Transverse Section through the strobilus axis in *Equisetum*

Typically vegetative shoots may bear cones in most of the species, but in some (e.g. *E. arvense*) two different types of branches are formed having different functions, i.e. some are sterile and green while other branches are non-green and fertile. The fertile branches in such species are short-lived and wither soon after spore dispersal.

Therefore, in general, the unbranched aerial shoot bears a terminal cone, but in some cases, branched fertile axis has also been reported.

Each strobilus or cone has a thick central axis called strobilus axis. On the strobilus axis are attached many umbrella-shaped sporangiophores. Each sporangiophore is a stalked and sporangia-bearing organ, the free end of which becomes flattened to form a **peltate disc**. The disc is a hexagonal structure arising at right angles from the axis. The flattened tip of the peltate disc provides a protective covering for the sporangia. On the undersurface of the disc are present many sporangia (Fig. 4 and 5).

Each sporangium is elongated pendant and sac-like structure having a rounded apex. The sporangia vary considerably in size, and their number varies from 5-10 in each sporangiophore.

The sporangia in *Equisetum* are of eusporangiate type in development. Smith has argued that this is mainly because "they are not entirely derived from a single initial cell".

Spores and Elaters

Beer (1909) has observed that the spores in *Equisetum arvense* and *E. limosum* are surrounded by a wall consisting of four layers, i.e. innermost cellulose layer called intine or endospore that remains surrounded by exine or exospore. The exospore remains surrounded by a cuticular layer called middle layer that is again covered by the outermost covering called perispore or episporium. The outermost perispore and the immediate next middle layer are laid down by the surrounding cytoplasm.

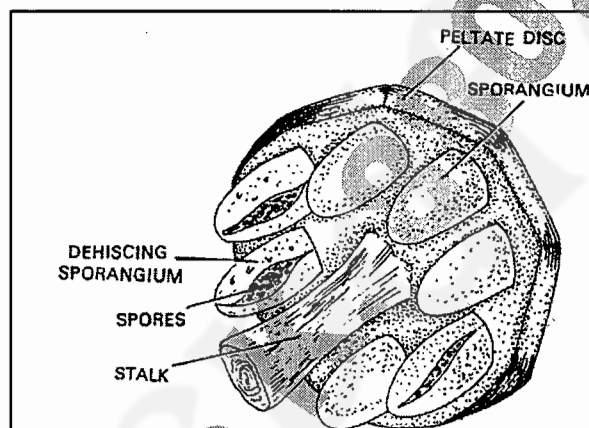


Figure 5: A single sporangiophore in *Equisetum*

Eames (1936) has mentioned that the outermost cuticular layer of perispore is differentiated into narrow, spirally wound bands with flat, spoon-like tips. These are called **elaters**. Four such bands are attached to a common point on the spore. The elaters are hygroscopic in nature and quickly respond to the environmental changes. In moist conditions, the elaters remain coiled around the spore but in dry conditions they become uncoiled and free, thus helping in spore release and dispersal.

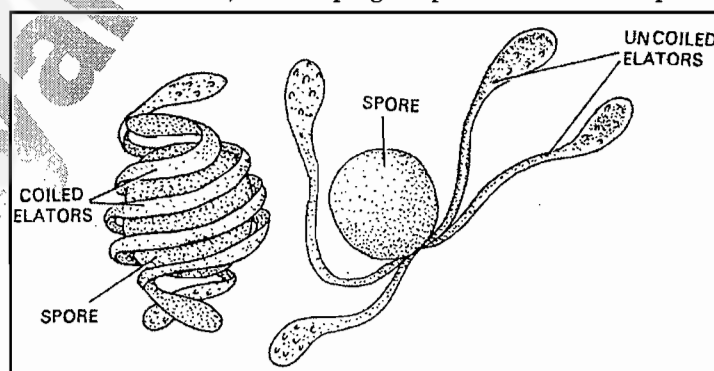


Figure 6: Spores with elaters in *Equisetum*

Possible Heterospory in *Equisetum*

Equisetum is morphologically and behaviorally homosporous. It implies that the gametophyte in *Equisetum* is bi-sexual in most cases.

However, McClean and Cook (1951) have shown in *E. arvense* that a distribution curve of the size indicates a bimodal structure showing a sign of a trend towards heterospory. According to them there is a difference of about 25% between the, size of smaller and larger spores of this species.

There are certain observations by botanists, which indicate some sort of shift in *Equisetum* species towards behavioral heterospory. It means that while the spores remain largely of the same size, they tend to germinate differently sometimes and give rise to unisexual prothalli under certain circumstances. This type of behaviour has been termed as **Incipient Heterospory**.

One such observation was made by Dr. Kashyap (1917), who noted that the prothalli of *E. debile* are small and bear only one kind of sex organ (either male or female) if the spores are sown in very close proximity. On the other hand, if the spores are spread with a sufficient space between them, the prothalli are large and bear first the archegonia and then the antheridia on the same prothallus. This indicates that whether this species is monoecious or dioecious depends upon the conditions. *E. arvense* is also a monoecious species but under unfavourable conditions, some of the prothalli develop only one type of sex organs, i.e. either archegonia or antheridia.

Spike in Ophioglossum

Ophioglossum

Ophioglossum (adder's-tongue) is a genus of about 25-30 species of Ophioglossales in the family Ophioglossaceae, with a cosmopolitan but primarily tropical and subtropical distribution. The name *Ophioglossum* comes from the Greek, and means "snake-tongue".

Ophioglossum has the highest chromosome count of any known living organism, with up to 1,400 chromosomes. However, most species only have chromosomes in the 240-300+ range.

Adders-tongues are so-called because the spore-bearing stalk (that is the **spike**) is thought to resemble a snake's tongue.

Each plant typically sends up a small, undivided leaf blade with netted venation and the spore stalk forks from the leaf stalk, terminating in sporangia.

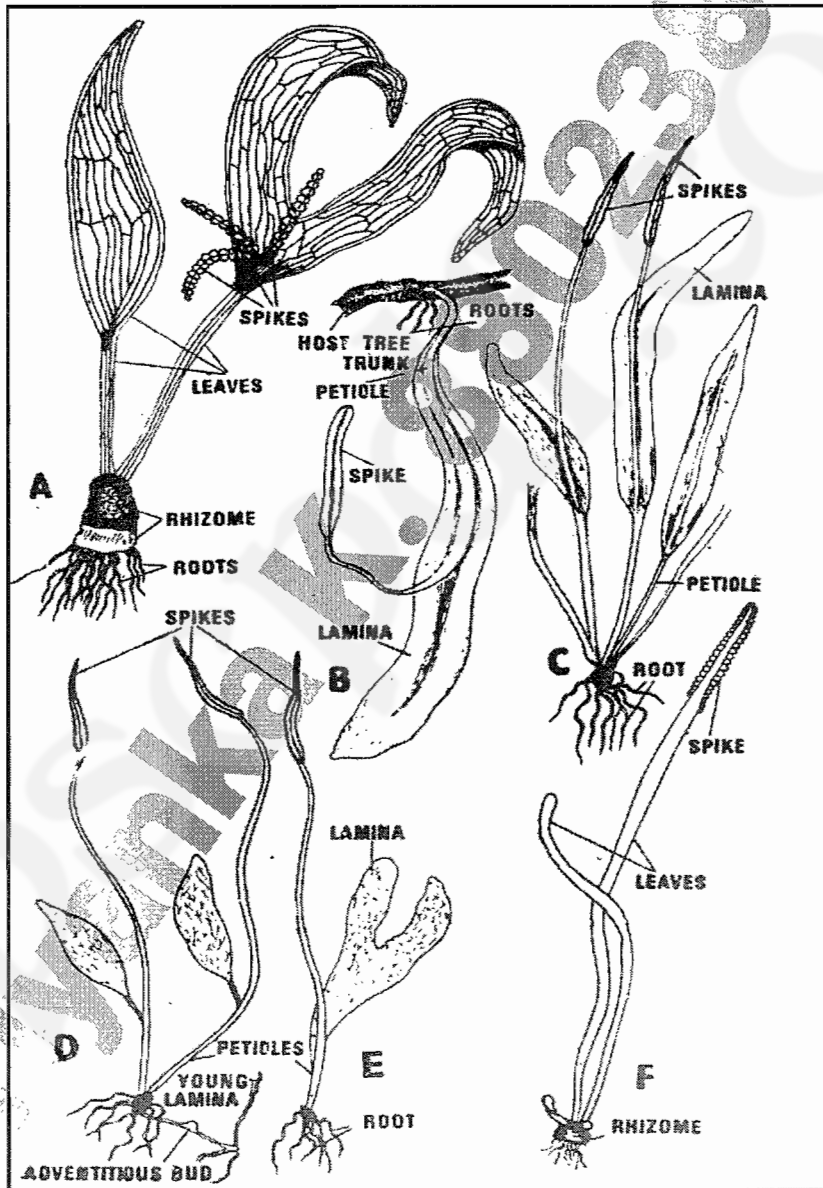


Figure 1: Morphology of various *Ophioglossum* species

The Fertile Spike

The spike is a specialized segment of the leaf. The mature spike has a lower sterile cylindrical portion called the stalk or the peduncle. The stalk is attached to the base of the lamina of the sterile leaf on its ventral side. The upper portion of the spike is broader and bears two rows of embedded sporangia on either side. The tip of the spike is sporangia free and somewhat conical in shape (Fig. 2). The spike varies in length in various species. In *O. aitchisoni* (Fig. 1 C) the spike is longer than the sterile leaf blade. In *O. pendulum* (Fig. 1, B) the spikes are shorter than the leaf blades. Usually one fertile spike arises from the leaf but in *O. palmatum* number of small spikes arise on either side of the petiole (Fig. 1, A). *Ophioglossum simplex* (Fig. 1, F) is characteristic in lacking a sterile lamina. The leaf, in this case, is long and cylindrical and terminates in a sporiferous area.

The free tip of the spike consists of parenchyma and vascular tissue. It no longer contributes to the growth in length of the spike. The sporangia originate during the early ontogeny of the spike when it is very small.

Peterson and Elizabeth Cutter (1967) attribute the growth in length of the spike due to an intercalary meristem present below the sporangial area. They removed the sporangia free tip of the spike and found that it did not affect the growth of the stalk or the peduncle. The peduncle continues in the sporangial region as a central axis and terminates in a small sterile and conical tip.

The sporangia are borne in two-distinct and longitudinal rows on either side of the axis (Fig. 2).

The sporangia are completely embedded in the tissue of the spike and their position is indicated by distinct transverse furrows of fertile tissue. These furrows alternate with the sporangia. The spike receives its vascular supply from the petiole. The vascular strands run upward and anastomose during their upward

course. They give out lateral strands that enter the sterile mass between the sporangia (Fig. 2). These lateral strands then bend towards the bases of the sporangia and constitute their vascular supply. The number of sporangia embedded in each spike varies from six to twenty or even more.

Structure of the sporangium: The completely embedded sporangium may be spherical or oval. It has a several-layered thick wall. The innermost wall layer functions as a tapetum. The cavity of the sporangium is filled with a compact mass of spore mother cells (Fig. 2-B, C, D). The tapetal cells disorganize and their cytoplasm forms a continuous mass of tapetal plasmodium with the several tapetal nuclei embedded in it.

There are numerous spores within the spore cavity. The number of spores may reach 15,000 per sporangium.

Due to the absence of any specialization in the sporangial wall there is no special mechanism concerned in the dehiscence of sporangial wall. A transverse slit appears in the sporangial wall and causes its dehiscence. The shrinking of the masses of sterile tissue in the spike may cause the dehiscence of the sporangium.

Bower (1896) studied the development of spike and sporangia in *Ophioglossum*. The spike originates as a small and a conical outgrowth of meristematic tissue. This outgrowth arises at the base of the lamina on the ventral side. Soon a pyramid-shaped apical cell appears in this meristematic tissue. This apical cell cuts off segments along its four sides and its activity leads to the formation of four quadrants, two of which lie parallel to the surface of the sterile lamina and the other two in a plane

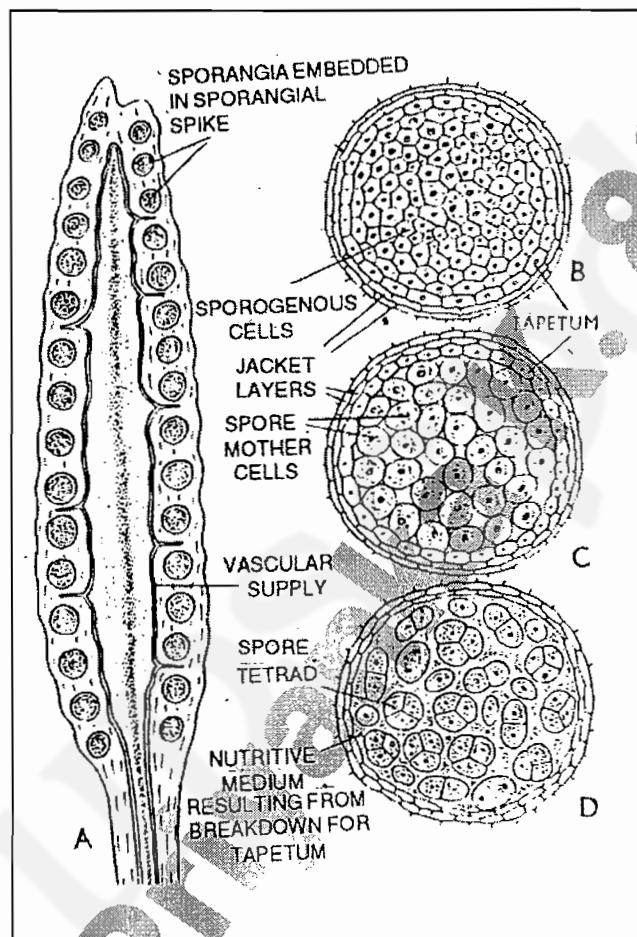


Figure 2: A. The spike in longitudinal section; and B, C, D: The spike in TS at different heights

perpendicular to it. Further growth and division of the segments of the apical cell lead to the formation of the spike.

Morphological Nature of the Spike

The following views have been expressed by various authors to explain the morphological nature of the spike in *Ophioglossum*.

1. Bower (1896, 1935) suggested that the simple spike of *Ophioglossum* originated as a result of condensation and lateral concrescence of the branched spikes of genera like *Botrychium*. He believed that the sporangia of the fertile spike are actually synangia derived by the partition or fission of the continuous sporangia. He believed the two marginal rows of sporangia to be two continuous sporangia, which have become partitioned to form two rows of sporangia. Such an assumption is not supported by the course of vascular supply from the petiole to the sporangia. Bower later (1926) abandoned his own hypothesis and agreed largely with Zimmermann (1930) in considering the spike to be a fertile lobe of the leaf.
2. Roeper (1859) considered the leaf of *Ophioglossum* to be derived from a compound leaf whose two basal pinnae or leaflets became laterally fused to form the spike. The remaining pinnae fused to form a sterile blade. Such an assumption is supported by the nature of the vascular supply to the spike. Goebel (1915) regarded the spike to be a modified single pinna of a leaf. Roeper's hypothesis was later upheld by Chrysler (1910) and Bower (1926).
3. Zimmermann (1930) suggested that the fertile spike and the sterile lamina are the two dichotomies of a shoot. The former is meant for reproduction and the latter for photosynthetic functions. The vascular supply from the petiole to the spike and the sterile leaf blade, no doubt, suggest that the spike is a modified segment of the leaf. A noteworthy feature in this connection is the growth of the spike. After the inception of the sporangiferous region the spike ceases to grow by means of an apical cell. It seems to elongate by the activity of an intercalary meristem (Peterson and Cutter, 1967) situated at the base of the sporangiferous region.

Adiantum

Occurrence

The genus *Adiantum* has 212 described species of which 36 species have been reported from India [Srivastava, 2001]. The species are widely distributed both in tropical and temperate regions of the world. All the species are terrestrial.

In our country, *Adiantum* spp are chiefly distributed in the Himalayan range. The common Indian species are *A. caudatum*, *A. capillus-veneris*, *A. lunulatum*, *A. edgeworthii*, *A. venustum*, *A. athiopicum*, *A. incisum*, *A. pedatum*, etc.

The genus is commonly called **maiden hair-fern**, because the plants produce leaflets similar to *Ginkgo* leaves and *Ginkgo* is popular as the maiden hair tree.

Morphology

The visibly dominant & ecologically persistent plant body is the diploid sporophyte. It is differentiated into root, stem and leaves (Figure 1).

The stem is modified into **subterranean rhizome**. It is usually long and creeping. Rarely, it is erect or semi-erect.

There are four outstanding features of the rhizome.

1. The leaves arise towards the upper side and the roots arise towards the lower side of creeping rhizome.
2. It is **perennial** and serves as an efficient means of vegetative propagation.
3. It is **dichotomously branched** even at maturity.
4. It is thickly covered with scales, called **paleae**. The paleae are flat, multi-seriate and generally lanceolate in shape.

The **adventitious roots**, which replace the primary roots when the plant matures, arise in clusters from the underside of creeping rhizome. The roots are branched, black in colour and wiry in texture. They also give out unicellular root hairs.

The leaves are compound. They can arise alternately or spirally. The young leaves are **circinately coiled** and slow growing. Each leaf has a **long, shining black petiole**, which is traversed by a median groove. In most of the species, the petioles are covered with paleae. In some cases, the petioles and rachis bear **multi-cellular non-glandular hair**.

The leaves are pinnately compound. The rachis of leaf varies in length from 3 to 40 cm in different species.

The leaves may be simply unipinnate (*A. caudatum*), bipinnate (*A. capillus veneris*) or 3-4 pinnate (*A. venustum*). In case of bi- or multipinnate species, the leaflets are called pinnules. The leaflets show dichotomous venation (Figure 2). The veins spread in fan-like manner in the lamina of leaflet.

Anatomy

Stem (rhizome)

A transverse section of rhizome shows almost circular outline. It is differentiated into epidermis, ground tissue and stele.

Epidermis: It is single layered, composed of thin-walled and smaller cells. The outer cell walls are brown and coated by thick cuticle. In some species, the epidermis bears multi-cellular hair.

Ground tissue: It is **parenchymatous** in composition. Sometimes, it may be preceded by 2-3 layered sclerenchymatous hypodermis. In certain species like *A. pectinatum*, *A. peruvianum* the patches of sclerenchymatous cells are scattered in the ground tissue.

Stele: A great variation in the structure of stele has been observed in different species of *Adiantum*. The stelar variation is represented by two major lines of organization.

1. In *A. rubellum*, the stele is **amphiphloic siphonostele**. It means that the vascular cylinder is siphon-like in which the ring of xylem is surrounded on both outer and inner sides by phloem pericycle and endodermis. The centre is occupied by pith. The arrangement of various tissues from periphery towards pith is → outer endodermis → outer pericycle → outer phloem → xylem → inner phloem → inner pericycle → inner endodermis.
2. In species like *A. capillus-veneris* and *A. caudatum*, formation of leaf trace and leaf gap breaks the cylindrical nature of siphonostele. It is further broken by many closely placed and overlapping leaf gaps

making it a dictyostele. In a dictyostele, several small meristels are arranged in a ring. It has outermost single layered endodermis followed by one or two layered pericycle. Next to pericycle is the zone of phloem in which the protophloem surrounds the metaploem. The protophloem consists of small sieve tubes and large parenchymatous cells. The metaploem consists of large sieve tubes. The companion cells are absent. The centre of meristele is occupied by xylem in which the protoxylem is surrounded on all the sides by metaxylem. The meristeleles are concentric in nature.

Reproduction

Reproduction by spores

The reproduction in sporophytic plant occurs by spores produced inside the sporangia. All the species of *Adiantum* are **homosporous** i.e., bear only one type of spores. The sporangia are borne in groups, called sori. They are borne on the reflexed outgrowths of fertile pinnules (**Figure 3**).

Sporophylls and sori

A fertile leaf bearing sporangia, is called sporophyll. In *Adiantum*, any vegetative leaf can develop sporangia and become a sporophyll. The sporangia are borne superficially all along the reflexed distal margin of fertile pinnule. The margin of pinnule blade is folded towards the lower side to form the **false indusium**. It covers the groups of sporangia called sori. The sori are **sub-marginal** in position.

They may be continuous in the form of **coenosorus** or in the form of small and separate sori.

In some species (*A. tenerum*, *A. rubellum*, etc.), the sori bear long, uniseriate, multi-cellular, hair-like paraphyses which are interspersed with the sporangia.

The development of sporangium is **leptosporangiate type** i.e., the sporangium originates from single superficial cell and the mature sporangium has single layered wall.

Spore formation

The sporogenous cell divides by four successive divisions to form **16 spore mother cells**. The cell walls of tapetal cells break down and form a multinucleate nutritive fluid. The spore mother cells separate from each other and float in the nutritive fluid. Each spore mother cell divides by meiosis and forms **4 spores** arranged in spore tetrad. Thus, about **64 spores** are formed which fill the enlarged cavity of sporangium.

Sporangium structure

The mature sporangium has two parts: **stalk** and **capsule**. (**Figure 3**)

The stalk is long, cylindrical, and consists of **three vertical rows** of cells.

The capsule is oval or biconvex-lens shaped, with a single layered jacket.

The single layered wall (Jacket) of sporangium shows a characteristic arrangement of cells.

- It shows the presence of a specialized band of thick walled cells called **annulus**. The annulus consists of vertical row of **12–24 narrow cells** which encircle about three fourth of the sporangium starting from the stalk at one side, running over the top and extending about half way to the other side. *The radial and inner tangential walls of annulus are quite thick but the outer tangential wall remains thin.* On one side, the annulus joins with the stomium.
- The **stomium** consists of about four transversely elongated thin-walled parenchymatous cells. Out of four stomium cells, the two upper ones which join the annulus are called epistomium and two lower ones which join the stalk are called hypostomium. The stomium is separated from annulus by **2–6 ordinary cells** whereas it is separated from stalk by **2–3 ordinary cells**.

Dehiscence mechanism

The dehiscence of sporangium normally occurs in dry weather. Drying causes outer cells of sporangium, including those of annulus, to lose water. The water is lost from the cells of annulus and their internal volume decreases. The outer thin walls of annulus cells are sucked-in so that the outer surface area decreases. This creates a tension in the entire annulus strip. It results breaking of stomium between the two lips, where the walls are extremely delicate. The annulus bends backwards rupturing the body of sporangium and carrying its upper half containing spores. The spores are dispersed in air.

As the cells continue to dry, the cohesive force of water within the cells of annulus decreases considerably. Their thin outer walls are pushed towards outer side. It results annulus to snap back to its original position. During this movement the spores are thrown away with a jerk to some distances and carried away by wind.

Sexual reproduction

The gametophytic generation begins with the formation of haploid spores. They germinate to produce gametophytic plant body.

The spore germinates as soon as it gets favourable moisture and temperature.

The prothallus (or gametophyte) is small (approximately 5 to 10 mm in diameter), green, heart-shaped (or cordate) and thalloid. It is flat, dorsiventral and shows an apical notch in which the growing apex is situated. The central portion of prothallus is several cells thick, called cushion whereas the lateral wings are one cell in thickness. All the cells are thin-walled, polygonal, uninucleate and bear many small discoid chloroplasts. The prothalli are autotrophic in their mode of nutrition. Many unicellular and unbranched rhizoids arise from the ventral surface near the posterior end. They penetrate into the soil and serve the function of anchorage and absorption.

Under favourable conditions the prothalli develop both the sex-organs (bisexual) on the ventral side. The antheridia are produced towards the base, among the unicellular rhizoids whereas the archegonia develop on the central cushion behind the apical notch.

The gametophytes of *Adiantum* are **monoecious** and bear both the sex-organs-antheridia and archegonia. The antheridia are borne towards the posterior side whereas the archegonia are borne upon the massive cushion, just behind the apical notch.

The mature antheridium dehisces in presence of external water. The antherozoids are liberated through the opening of antheridium. The antherozoids are spirally coiled and multi-flagellated. They swim in the film of water and reach archegonia.

The **archegonia** are female sex-organs. They are produced on the ventral surface of central cushion behind the apical notch.

Fertilization occurs in presence of water. The antherozoids are chemotactically attracted towards the open mouth of archegonia. They reach the archegonial mouth and enter into the neck canal. Many antherozoids can enter but only one fuses with the egg. The haploid nucleus of antherozoid fuses with the haploid nucleus of egg and forms diploid zygote. Usually only one archegonium is fertilized, the others remain unfertilized.

Zygote is the first cell of sporophytic generation. It remains embedded in the archegonium and develops into an embryo, which ultimately gives rise to mature sporophytic plant body.

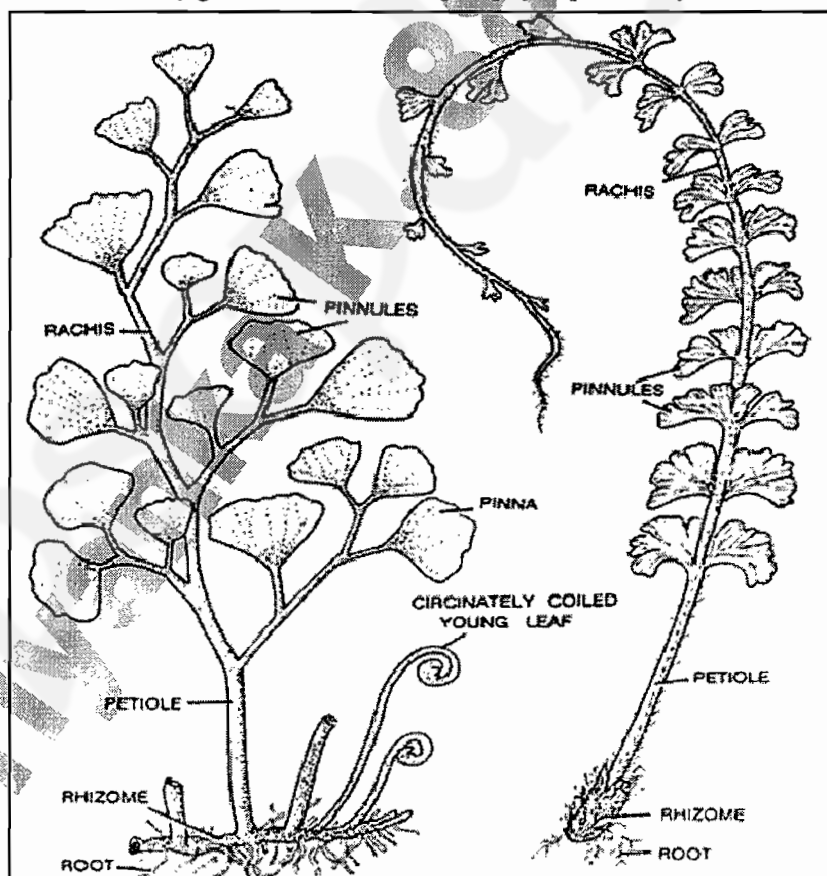


Figure 1: *Adiantum*: General Morphology

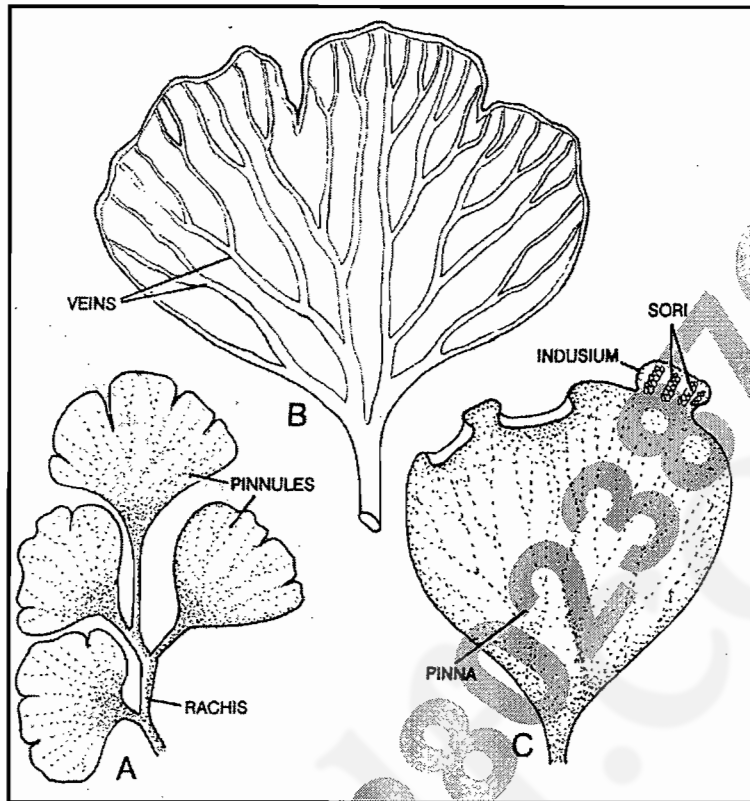


Figure 2: A pinna in *Adiantum*.

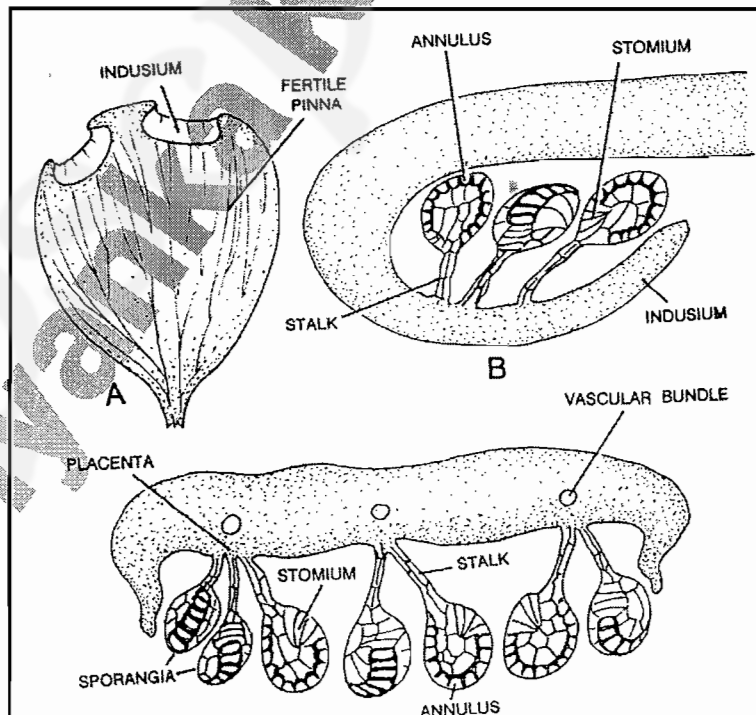


Figure 3: Sorus in *Adiantum*

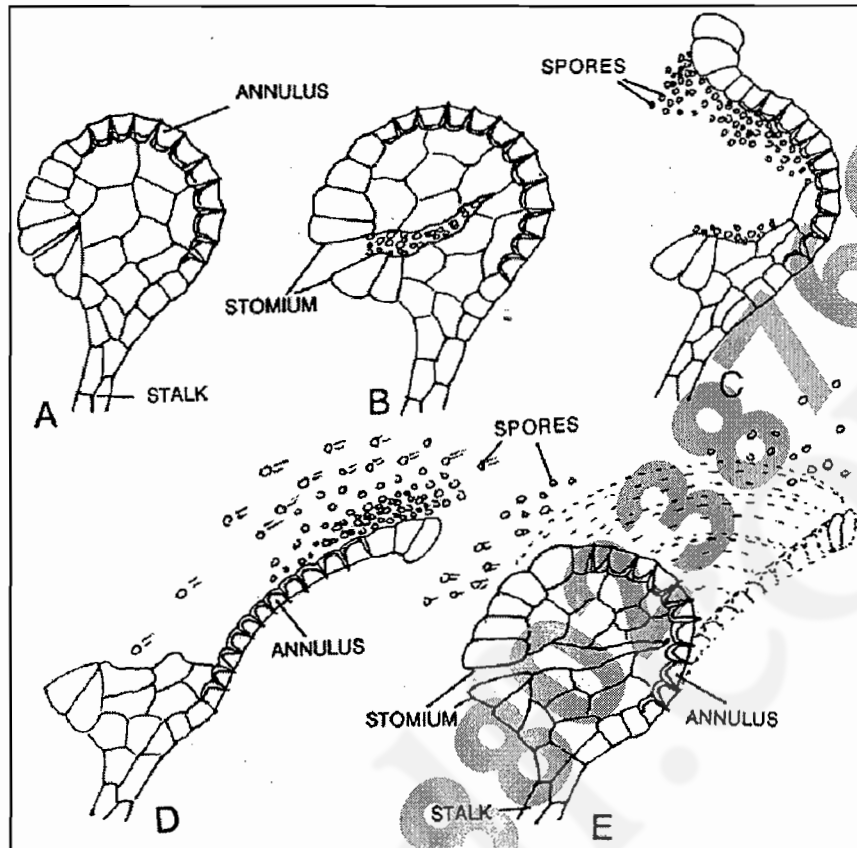


Figure 4: Sporangial dehiscence mechanism in *Adiantum*

Marsilea sporocarp

Marsilea

Plants of *Marsilea* are distributed worldwide, both in temperate as well as in tropical regions, most of the species being aquatic or semi aquatic hydrophytes growing in temporary ponds, pools and puddles after the rains. They grow partially or entirely submerged in water with their roots in the soil or mud and produce sporocarps under water.

The sporophytic plant body is mainly concerned with the production of spores, the haploid cells which develop into gametophytes. *Marsilea* genus is heterosporous. Both micro- and megaspores are produced in micro- and mega sporangia respectively and the sporangia are borne in special reproductive bodies called sporocarps.

The sporocarps

The sporocarps are special bisporangiate fruiting bodies i.e., produce two different kinds of spores-microspores and megaspores inside the microsporangia and mega sporangia respectively. (Lecture illustration 2)

Plants start producing sporocarps at a certain stage of maturity. The sporocarps are borne laterally on the adaxial side of petiole near the base. They are attached by means of short or long stalks called peduncles or pedicels. The mode of attachment of peduncles and their groupings vary in different species. (Lecture illustrations 1 and 3).

Each sporocarp is small, oval or oblong in shape resembling a bean seed. When young, they are soft, green and covered with small hairs but at maturity they become hard, brown and nut-like.

The place where peduncle meets the body of sporocarp is called raphe, particularly in those species where the apex of peduncle unites laterally with the posterior part of body. The sporocarp beyond the raphe bears one or two horn-like tubercles or teeth on its dorsal side. They are situated along the median line. Both the tubercles may be equally prominent (e.g., *M. minuta*) or one of them may be prominent and the other suppressed. In *M. quadrifolia*, the lower tubercle is stout and prominent whereas in *M. aegyptiaca*, the upper one is prominent. One or both the tubercles may be absent in some cases.

Internal structure of sporocarp (Lecture illustration 6)

The sporocarp is more or less bilaterally symmetrical body having two equal halves (valves). It has a very thick and hard wall. The wall consists of three layers. Outer single layered epidermis consists of small columnar cells. It is interrupted by stomata. The epidermis is followed by two layered hypodermis (or palisade) consisting of radially elongated cells. The upper hypodermal layer has thick-walled cells whereas lower layer has thin-walled cells. All the cells of hypodermis (palisade) contain chloroplasts. Just below the wall, there is a gelatinous ring encircling the central cavity of sporocarp in dorsiventral plane it is formed from parenchymatous cells which get gelatinized at maturity. This ring is more prominent in dorsal sides as compared to ventral side.

The central cavity of sporocarp encloses a group of closely packed elongated sori arranged in two rows, one row in each half (valve). The sori of the two rows alternate with each other. Each sorus is elongated in dorsiventral plane and placed transversely to the long axis of sporocarp. The number of sori in each sporocarp varies from 2-20 (e.g., 2 in *M. aegyptiaca*, 11-12 in *M. minuta*, 20 in *M. quadrifolia* and *M. vestita*). Each sorus arises on a ridge-like placenta (or receptacular ridge) developed on the sporocarp wall.

Characters of water and land plants of Marsilea

Species of *Marsilea* grow in wide varieties of habitats. Some are hydrophytes and grow partially or entirely submerged in water. Some are xerophytes and grow on dry lands. A few species usually grow in water but produce sporocarps under dry terrestrial conditions (amphibious in nature). They show variations in their morphology under different growing conditions. The plants growing in water show some special characteristic features than those growing under dry terrestrial conditions.

These are listed below.

Characters of water forms

1. The internodes of stem (rhizome) are very long.
2. The roots are unbranched.
3. The petioles are extremely long, weak and flexible. The leaflets float upon the water surface.
4. Sclerenchymatous tissues are practically absent in vegetative parts.
5. There are large air-spaces in all vegetative parts.
6. The stomata are restricted largely to upper surface of leaflets.

Characters of land forms

1. The internodes of stem (rhizome) are short.
2. The roots are much branched.
3. The petioles are short and erect with spreading leaflets.
4. Sclerenchymatous tissues are more in vegetative parts, particularly in the cortex and pith.
5. There are a few and small air-spaces.
6. The stomata are distributed on both the surfaces of leaflets.

It is surrounded by its own delicate, two-layered indusium. The elongated receptacular ridge bears a row of short-stalked mega sporangia along its top and a large number of long-stalked microsporangia along its sides. The mega sporangia and microsporangia of each sorus are enclosed within the soral indusium. The microsporangia are always more in number as compared to mega sporangia in each sorus. Each sporocarp is characteristically supplied by vascular traces.

Vascular supply of sporocarps (Lecture illustration 5)

Single vascular bundle transverses through the stalk (peduncle) and runs directly into the raphe, where it bends near the lower tooth and passes to the dorsal region of sporocarp. Here it is called dorsal bundle. The dorsal bundle then bifurcates into two branches near the apex and each branch enters into respective valve. The dorsal bundle or its two branches give off rib-like lateral branches which run back to each sorus. The lateral branches of each valve alternate with each other and their number corresponds to the number of sori. The lateral branches, during their course downward, fork dichotomously nearly in the centre. The apices of these branches anastomose with each other and with their corresponding branches of opposite valves forming a network. A small branch is given off from the point of dichotomy from each lateral branch which runs into the receptacle, known as receptacular or placental branch. The placental branch forks and the two placental bundles run dorsally and ventrally into the receptacular ridge.

Internal structure as seen in section of sporocarp cut in various planes

Vertical transverse section (V.T.S.) of sporocarp (Lecture illustration 8)

1. The wall of sporocarp consists of three layers. Outer single layered epidermis consists of broad and columnar cells. It is interrupted by a number of stomata. The inner two layers constitute the hypodermis (or palisade). The upper hypodermal layer has radially elongated thick-walled cells whereas lower layer has more elongated thin-walled cells. (Lecture illustration 4)
2. The palisade cells are green and possess chloroplasts.
3. The gelatinous ring is cut at two places and seen in the form of two gelatinous masses one on either side of the section. The upper gelatinous mass is bigger and more prominent than the other (towards ventral side). The receptacles are cut longitudinally.
4. Only two sori are seen in this plane, sometimes one bigger and one smaller. Each sorus is covered by its own two layered indusium. Inside the sorus, only 2-3 microsporangia are seen at the corners whereas a number of mega sporangia are linearly attached all along the receptacular ridge.
5. Dorsal bundle, lateral bundle, placental bundles and placental branches are seen in this section.

Vertical longitudinal section (V.L.S.) of sporocarps (Lecture illustration 9)

1. The section shows similar structure of wall as seen in vertical transverse section.
2. The wall is followed by a complete gelatinous ring, which surrounds the central cavity enclosing sori. The ring is more prominent towards dorsal side than the ventral side.
3. The sori are cut longitudinally and they are arranged in a row.
4. If the section passes somewhat to one side of the median line, only mega sporangia are seen inside the sori. If the section passes away from the median line, only microsporangia are seen arranged on either side of receptacle.
5. Each sorus is enclosed within a two layered indusium.
6. The stalk bundle and the cut lateral bundles are seen in this plane of section.

Horizontal longitudinal Section (H.L.S.) of sporocarps (Lecture illustration 7)

1. The section shows similar structure of wall as seen in vertical transverse section.
2. The stalk (penduncle) of sporocarp is cut transversely that shows the presence of stalk bundle.
3. Gelatinous ring is cut at two places and visible in the form of two gelatinous masses. The gelatinous mass towards stalk is much bigger and prominent than on the other side.
4. All the sori of sporogonium are cut transversely in this plane of section. They are arranged in two rows.
5. The sori of two halves alternate with each other. Each sorus is covered by its own indusium. The sorus shows single mega sporangium located centrally and microsporangia on either side of the receptacle.
6. Lateral bundles are cut transversely. The dorsal bundle, lateral branches and receptacular branches are seen in this section.

Morphological nature of the sporocarp (Lecture illustration 10)

The morphological nature of sporocarp of *Marsilea* has been a widely debated problem among various workers. However, different views of various workers can be grouped into following two heads for the convenience of study.

1. **Leaf segment or Laminar theory** (Campbell, 1905 Bower, 1926),
2. **Whole leaf theory** (Johanson, 1898, 1933).

Leaf segment or laminar theory.

According to this theory the sporocarp of *Marsilea* is regarded as a modified fertile segment of a leaf and can be derived from fertile pinna or leaflet. This theory has been supported by a number of workers. Views of these supporters are given below.

1. **Russow (1872) and Busgen (1890):** The sporocarp is composed of two leaflets with their ventral surfaces facing each other. In support of his view Busgen examined the abnormal leaf of *M. hirsuta* showing conditions transitional to sporocarp.
2. **Bower (1926):** He considered sporocarp to be rachis bearing two rows of pinnules. He derived this conclusion on the basis of venation. Bower has drawn a parallel between marsileaceae and Schizaeaceae, especially *Schizaea rupestris*, where the sponophyll has 6-10 serrated pinnules on either side. Lateral fusion of such pinnules would give a body similar to sporocarp, but oriented adaxially instead of abaxially.
3. **Eames (1936):** He regarded that sporocarp can be compared morphologically with the tip of leaf with four leaflets. According to him the two distal leaflets form the body of capsule and the proximal leaflets represented by the hump and two teeth (or tubercles).
4. **Smith (1955):** He considered the sporocarp of *Marsilea* with an enfolded leaflet having single leaflet and 15-20 lateral veins on either sides. He compared it with the enfolded pinna of a Cyatheaceous fern, which bears sori on abaxial surface covered by involucre like indusia.
5. **Puri and Garg (1953):** They regard sporocarp to be equivalent to a single leaflet. The number of commissural bundles (lateral bundles) correspond to the number of pinnules in a leaflet.
6. **KM. Gupta (1962):** He regarded the sporocarp to be a leaflet with as many lobes as the number of commissural bundles (lateral bundles). In his opinion the pinna is only lobed and not divided into pinnules as regarded by Puri and Garg.

Evidences in support of leaf segment or laminar theory

1. The vascular supply to the sporocarp and the vascular supply to the leaflet of sterile leaf show close similar-ties. This indicates that sporocarp is a modification of a leaf segment (leaflet) rather than an entire leaf. Such a similarity has been observed in *M. quadrifolia* by Eames (1936) and in *M. minuta* by Puri and Garg (1953).
2. Johnson (1898) reported that the second sporocarp develops from the marginal cell of the first and the third sporocarp develops from the marginal cell of the second. The second and third sporocarps are thus, regarded as the branches of first. This indicates that each sporocarp is actually a segment (branch) of leaf and not the entire leaf.
3. Certain abnormalities have been recorded by Busgen (1890) in *M. hirsuta* which support the leaf segment theory. He observed that some plants of this species bear leaves whose leaflets get modified into sporocarp like structures.
4. Occurrence of 10-15 sporocarps on one side of petiole as a series in *M. polycarpa* presents a strong evidence in support of leaf segment theory. Each sporocarp is compared with the pinna of once compound frond similar to *Pteris semipinnata*. In this case the pinnules are present only on one side.

Petiolar or whole leaf theory

Johnson (1933) regards the sporocarp to be swollen end of sterile leaf branch (petiole) in which the marginal cells act as sporangial initials instead of acting as initials for four leaflets. The leaf and sporocarp both show a similar mode of their development from single initial cell with two cutting faces whereas pinna develops from marginal cells with five cutting faces. Another point in favour of whole leaf theory was his explanation regarding the nature of secondary and tertiary sporocarps. He could explain it by assuming bi- or tripinnate compound leaf.

Sporocarp regarded as a new organ

Bierhorst (1971) regarded the sporocarp of *Marsilea* as a new organ having sporangial ontogeny in early stage and pinna ontogeny in later stage.

Sporangia

The genus *Marsilia* is heterosporous and produce two kinds of spores—the microspores and the megaspores. The microspores are produced inside the microsporangia, usually 32–64 in each microsporangium. The megaspores are produced single in each mega sporangium. Both kinds of sporangia are borne on the receptacle in a basipetal succession. They are aggregated in sorus which is covered by its own indusium. The sori are located within the sporocarp and liberate only when the later dehiscence.

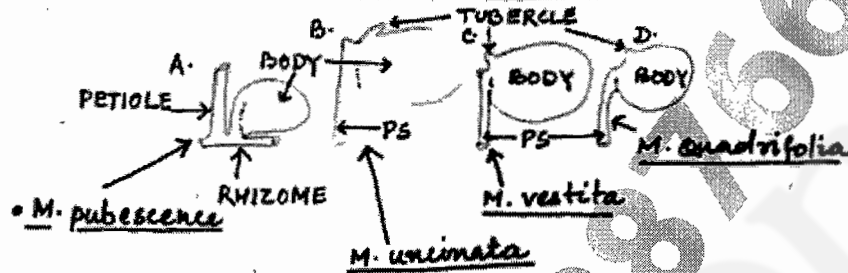
The outer rind (wall) of sporocarp is very hard and resistant to mechanical injury and drying. It may remain undehiscent upto 2–3 years unless opened artificially. Under natural conditions probably it is opened due to partial decay of the outer hard wall. The spores remain viable and can survive upto 20–30 years (sometimes upto 50 years).

If the ventral margin of a mature sporocarp is slightly ruptured artificially and placed in water, the water enters through the slit and the gelatinous material starts swelling. Within 15–20 minutes the sporocarp wall splits into two valves and the gelatinous ring starts pushing out. Due to imbibition of water the gelatinous ring swells and greatly expands so that it is pulled out of the slit in a looped condition. The sori, attached to gelatinous tissue, are also pulled out along with the ring. The ventral end of gelatinous ring gets detached from the sporocarp and it begins to uncoil in a worm-like manner. Finally the gelatinous ring becomes straight bearing two rows of sori, one on either side. The sac-like sori then get separated from gelatinous material. The indusia of sori also imbibe water and become gelatinous, with the result the ventral end of sori torn open and some of the sporangia containing spores escape from the open end. Finally the wall of sporangia comes in contact with water, become mucilaginous so that the mature spores are liberated. The spores germinate after liberation. The whole process of spores' liberation from the sporocarp, under artificial condition, takes about 5–6 hours.

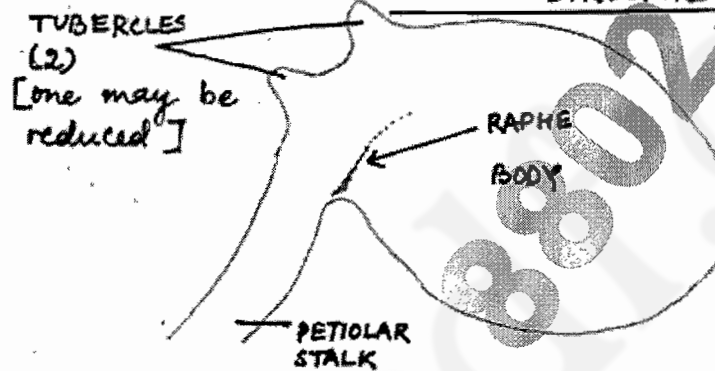
Illustrations

MARSILEA SPORO-CARP-①

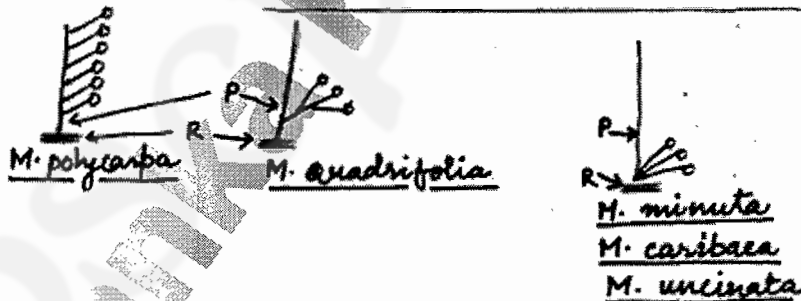
1. OUTLINE OF THE STRUCTURE: VARIATIONS.

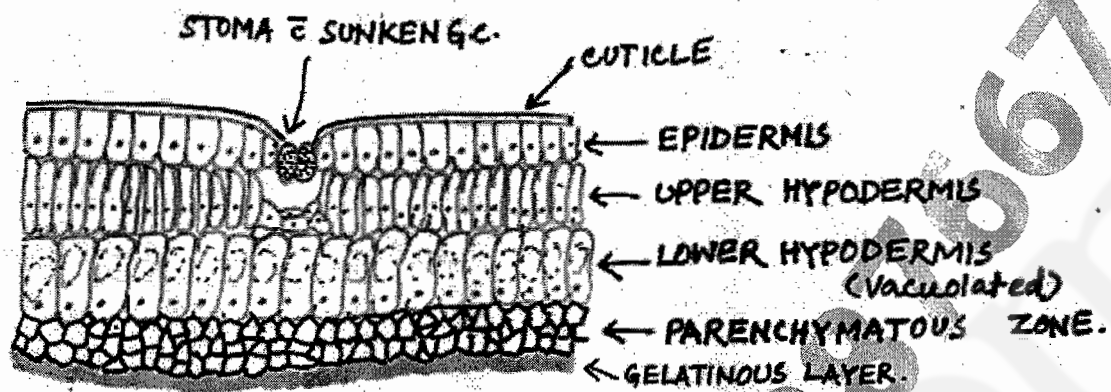


2. A GENERALIZED COMPOSITE STRUCTURE

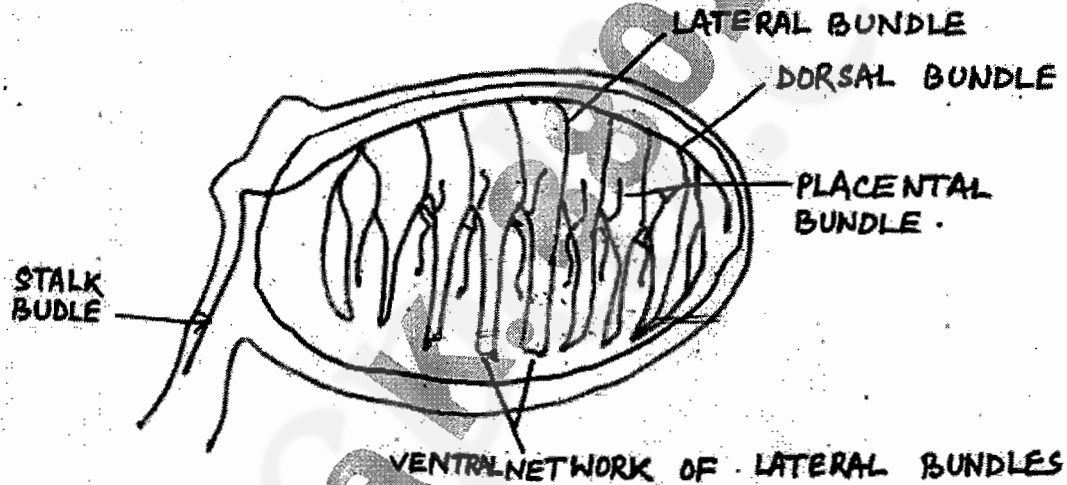


3. ATTACHMENT TO THE AERIAL SHOOT.

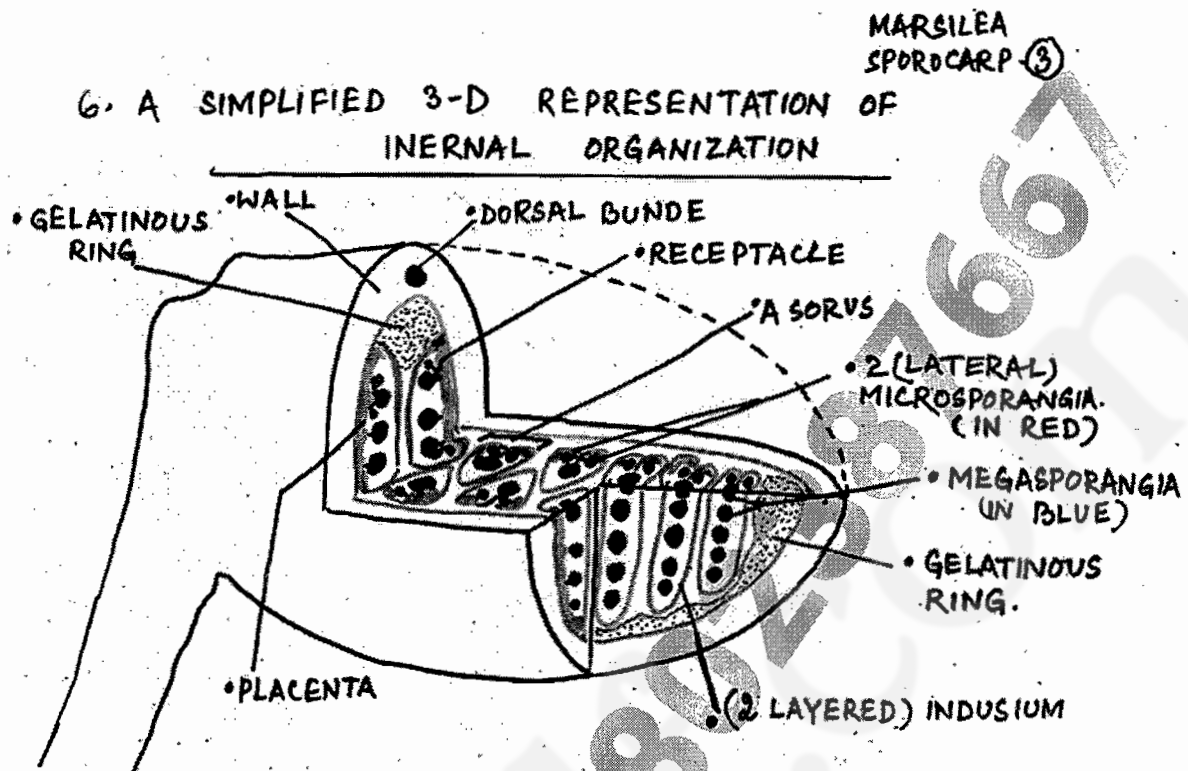




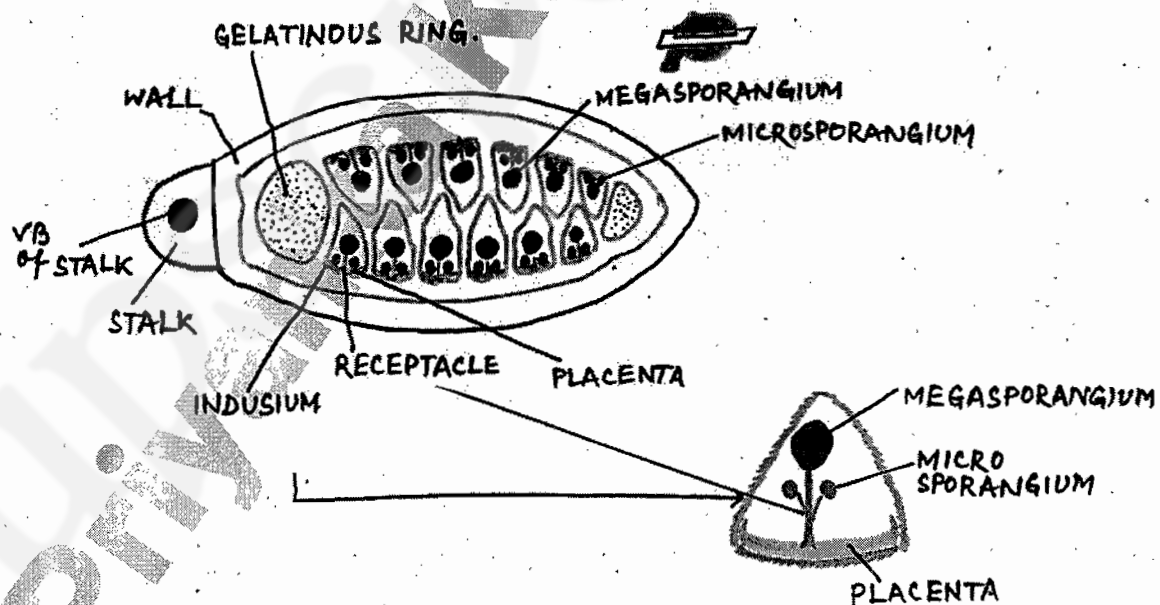
4. VARIOUS WALL LAYERS OF SPORO CARP



5. VASCULAR SUPPLY.



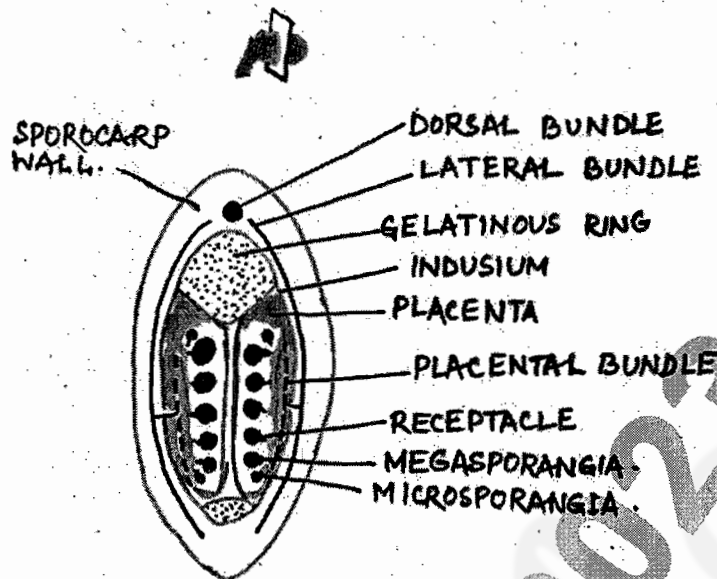
7. HORIZONTAL LATERAL SECTION



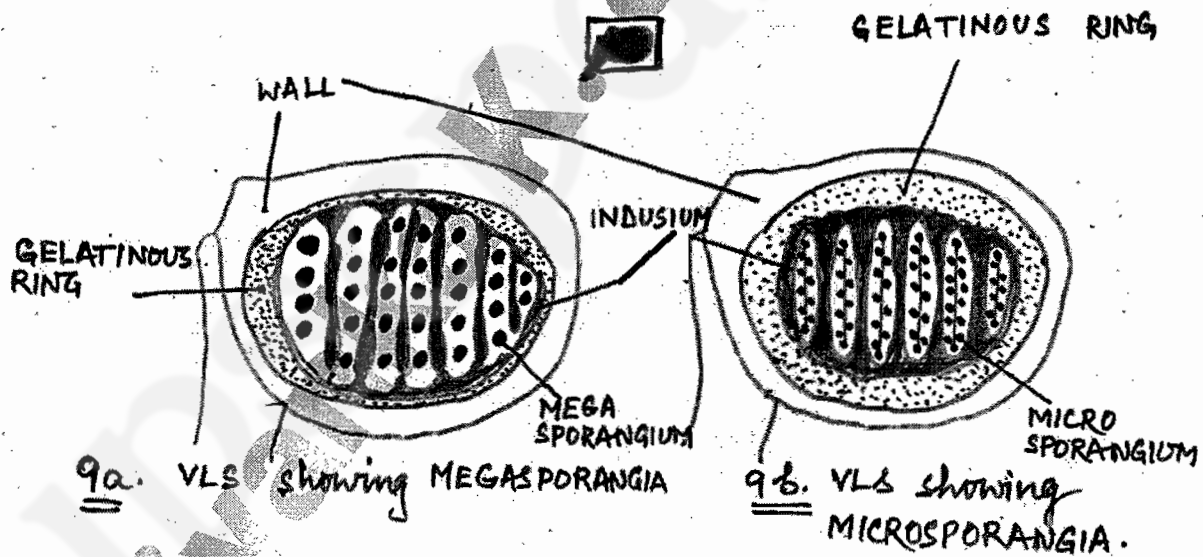
7a. A SORUS AS SEEN
IN H.L. SECTION.

MARSILEA: ④

8. SPOROCARP IN VERTICAL TRANSVERSE SECTION.

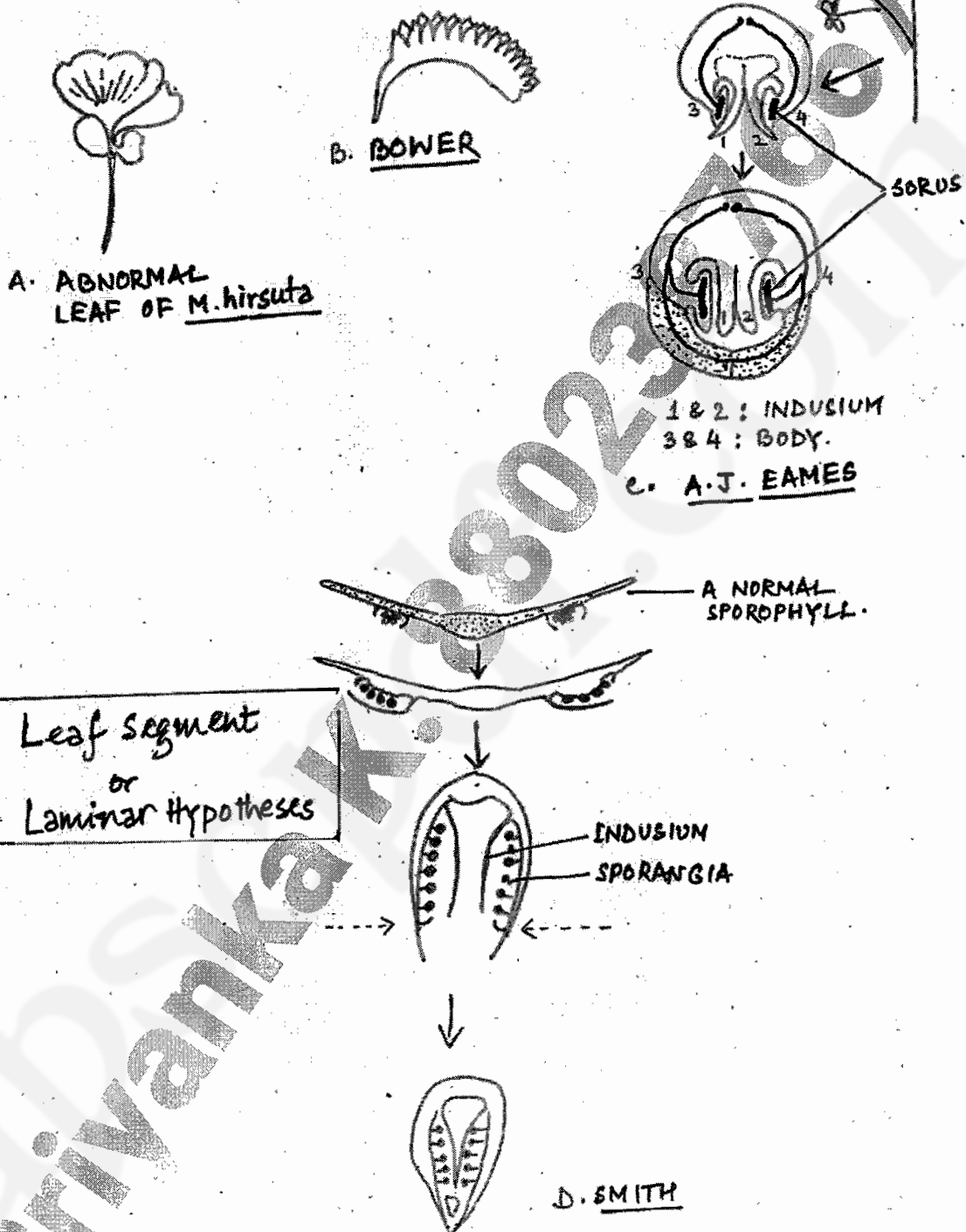


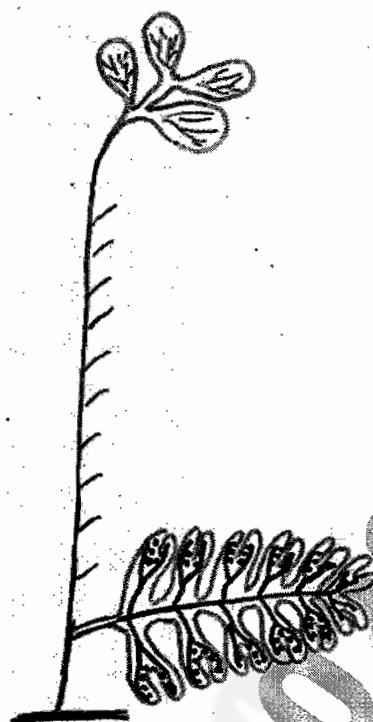
9. SPOROCARP IN VERTICAL LONGITUDINAL SECTION.



5 Marsilea

VARIOUS VIEWS ON MORPHOLOGICAL NATURE OF MARSILEA SPORO-CARP





e. PURI & GARG

Apospory and Apogamy in the Pteridophytes

Apospory

Production of non-vascular gametophyte directly from the vegetative cells of the vascular sporophyte, without the formation of spores is called apospory. The phenomenon occurs in nature, as well as can be induced artificially. The gametophytic prothalli, produced as a result of apospory, possess the same chromosome number as the sporophyte (i.e., diploid or $2n$). They behave in the normal way and develop sex organs (antheridia and archegonia) and the gametes. The gametes produced in such prothalli are also diploid. Their fusion results in the formation of tetraploid ($4n$) zygote, which grows to produce tetraploid sporophyte. Thus, apospory may be regarded as one of the pathways inducing natural polyploidy in pteridophytes.

The phenomenon of natural apospory was first reported by Druery (1884) in fern called *Athyrium filix-femina* var. *clarissima*, where the gametophytic prothalli were formed from the stalk of sporangium. Since then it has been reported in a number of pteridophytes, particularly ferns. Many workers have also been able to induce apospory in a number of ferns under specific culture media and environmental conditions. Briston (1962) developed gametophytic prothalli from a callus tissue derived from the sporophyte of *Pteris cretica* by supplying the specific nutritional medium in the culture. Takahashi (1962) successfully induced apospory in *Pteridium aquilinum* var. *latiusculum*. Charles Morlang (1967) successfully induced apospory in three species of *Asplenium* (*A. platyneuron*, *A. rhizophyllum* and *A. montanum*) from cultured leaf segments. Palta (1973) induced apospory in *Pteris vittata* from young leaves under specific culture conditions. Munroe and Bell (1970) studied various steps in the induction of apospory in the root cells of *Pteridium aquilinum*. The root cells were cultured in "starvation medium" and observed the structural changes. They observed disorganization of root meristem, degeneration of some root cells, transformation of remaining viable cells and differentiation of gametophyte.

The various factors which influence the development of aposporous gametophytes include the nutritional and biochemical status of the medium, low light intensity and the age of sporophytic cells. It has been found that complete absence of carbohydrate in culture medium (called starvation medium) induces development of aposporous gametophytes in *Pteridium aquilinum*. Low light intensity favours formation of aposporous prothalli in *Dielyria rigidula*, *Polypodium aureum*, *Platycenum bifurcatum* and *Platycenum grande*.

Apogamy

Production of vascular sporophyte directly from the vegetative cells of non-vascular gametophyte, without the act of syngamy or gametic union, is called apogamy (Winkler, 1908). According to the definition the apogamous sporophytes are always produced from vegetative cells, but not from eggs. If the egg develops into sporophyte without fertilization or syngamy, it is called parthenogenesis. The phenomenon of apogamy was observed first by Farlow (1874) in *Pteris cretica* and termed apogamy by De Bary (1878).

Apogamy occurs in nature, as well as can be induced artificially under controlled conditions. The sporophytes, produced as a result of apogamy, possess the same chromosome number as the gametophyte. Initially they originate from a group of two or more cells in the thickened part of prothallus. First division is always periclinal and the subsequent 2-3 divisions are in the same manner as gametophyte. Later, they develop small chloroplasts and form an apical meristem of sporophyte. Shoot and leaf apices originate in the meristem which begin to develop stem and leaf. The first root arises adventitiously from the basal end of stem. The xylem elements differentiate acropetally as soon as the shoot and leaf apices start functioning. The apogamous embryo develops in a normal manner and produces a mature and comparatively large sporophyte.

Natural apogamy has been reported in more than 50 species of ferns belonging to 20 genera and 4 families. Some of them are *Pteris*, *Dryopteris*, *Adiantum*, *Osmunda*, *Asplenium*, etc. However, apogamy has been induced artificially in a number of ferns and a few species of *Lycopodium* and *Equisetum*.

The nuclear behaviour in the life-cycle of obligate apogamous ferns is most characteristic and do not follow the normal course (i.e. alternation of $2n - n$ generations). These ferns maintain the same level of chromosome number in sporophyte as well as gametophyte. In these ferns, doubling of chromosome number (i.e., Syndiploidy) occurs prior to meiosis or reduction division. For example, a diploid sporophyte ($2n$) develops tetraploid ($4n$) spore mother cells by the process of syndiploidy. The tetraploid spore mother cell divides by meiosis and forms diploid ($2n$) spores. These spores germinate to produce diploid gametophytes which develop diploid apogamous sporophytes.

The various factors which influence the development of apogamous sporophytes include the nutritional and biochemical status of the medium, light quality and growth hormones. It has been shown experimentally that apogamous sporophytes are produced if the gametophytes are grown in 4% sucrose solution at the initiative phase. It has also been shown that far-red light (705 μm) followed by blue (445 μm) and white light is most effective in inducing apogamous development of sporophyte. Formation of apogamous sporophytes is stimulated in presence of growth hormones such as gibberellic acid (GA_3), indole 3-acetic acid (IAA), ethylene, etc., in the culture medium.

Parthenogenesis

Development of embryo (sporophyte) from egg without fertilization or syngamy is called parthenogenesis. The phenomenon of parthenogenesis was reported for the first time by Farmer and Digby (1907) in three ferns — *Athyrium filix-foemina*, *Athyrium filix-foemina* var. *uncoglomeratum* and *Scolopendrium vulgare*. Since then, the phenomenon has been reported to occur in many pteridophytes such as many species of *Selaginella* (*S. spinosa*, *S. nibicaulis*, *S. helvetica*, *S. anocardia*).

Diversity and distribution pattern of Pteridophytes in India

Introduction

The vascular flora of our country in general has about 15,000 species and as a constituent of Indian flora of vascular plants, the ferns and fern-allies form only five percent part as far as the number of species is concerned.

Pteridophytes form an interesting and conscious part of our national flora with their distinctive ecological distributional pattern.

On a very conservative estimate 500 species of ferns and 100 species of fern-allies are on record from India. According to a census, the Pteridophytic flora of India comprises of 70 families, 192 genera and more than 1,000 species (Dixit 1984).

Diversity and Distribution

There are about 12,000 species of pteridophytes occurring in the world flora, of which 1,000 species into 70 families and 192 genera occur in the different parts of the present Indian political boundary.

Region-wise studies reveal that:

- Maximum number of 700 species (i.e. 58% of Pteridophytes) occur in Eastern Himalayas and adjoining states. Thus, Eastern Himalayas may be termed as one of the Hot Spots diversity centre for pteridophytes.
- In other regions,
 - 400 species in Southern India
 - 300 species in North-West India
 - 100 species in Central India
 - 125 species in Andaman and Nicobar Islands.
- The maximum diversity has been observed between 1,200–2,800m. alt. in Temperate Himalayas and adjoining forest areas.

The maximum number of 150 species and 28 genera are in the family **Polypodiaceae**.

Afterwards, important families are **Dryopteridaceae** (109 species, 4 genera), **Athyriaceae** (101 species, 13 genera), **Thelypteridaceae** (88 species, 21 genera), **Aspleniaceae** (70 species, 4 genera) and **Aspidiaceae** (50 species, 11 genera).

Table 1: Comparative number of members of ten well representative families in Eastern Himalaya

Sl. No.	Name of the Family No. of species in India	Approximate no. of species in India	Approximate no. of species in	Percentage in Eastern Himalayas
1.	Polypodiaceae	150	107	71.3
2.	Dryopteridaceae	109	92	82.56
3.	Athyriaceae	101	92	75.24
4.	Thelypteridaceae	88	64	72.72
5.	Aspleniaceae	70	33	47.1
6.	Selaginellaceae	62	27	43.55
7.	Pteridaceae	60	43	71.66
8.	Aspidiaceae	50	23	46.0
9.	Sinopteridaceae	38	23	60.2
10.	Hymenophyllaceae	28	22	78.57

Endemic Pteridophytes of India

Of 530 Pteridophytes reported as endemic to the India in recent decades (about half the total number of 950–1000 known Indian species), only 47 endemic Indian ferns, less than 10% of those reported previously, are accepted by Fraser.

The great majority of endemic Indian Pteridophytes are peninsular-Indian to south-Indian ferns (27) with far fewer being N.E. Indian (7) and W. Himalayan (2); the floristically Malesian Nicobar Islands have (3).